

INTESTINAL GROWTH AND ADAPTATION IN GROWING ANIMALS
THE EFFECT OF AGE, LUMINAL NUTRITION,
GROWTH FACTORS AND ANTIGEN TRANSPORT

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MASTER OF SURGERY
University of Edinburgh 1983



ABSTRACT OF THESIS (Regulation 6.9)

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Degree MASTER OF SURGERY Date MAY, 1983

Title of Thesis INTESTINAL GROWTH AND ADAPTATION IN GROWING ANIMALS: THE EFFECT
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After major intestinal resection in the suckling and the immature rat, the residual intestine undergoes a marked compensatory regeneration, accelerated mucosal maturation and functional adaptation. The regeneration includes increases in villus height, crypt depth, marked cellular hyperplasia and cellular hypertrophy. The accelerated maturation includes early increases in sucrase activity, and the early appearance of effective exclusion of enterically presented antigenic bovine serum albumin. In the suckling rat, functional adaptation is characterized by increases in sucrase and lactase activity per centimeter and per cell.

The cellular hypertrophy and the increased sucrase activity per cell are in distinct contrast to the decreased cell size, and the decreased enzyme activity per cell, observed after intestinal resection in adult animals. Accelerated mucosal maturation may be partly responsible for both. The rise in lactase activity, however, represents a functional adaptation, because in weanling and weaned rats lactase activity per cell falls after enterectomy.

When food is absent from the intestinal lumen, compensatory regeneration is inhibited, normal maturation of structure and function fails to occur, and normal longitudinal and mucosal growth of the intestine are both reduced. Nevertheless, the short-term inhibition of the structural and functional response to intestinal resection, brought about by 10 days of parenteral nutrition, is only temporary, and is reversed by the reintroduction of normal feeding.

Salivary growth factors and gastrin do not aid in the compensatory response, and gastrin may even inhibit maturation of mucosal enzyme function. Growth factors present in breast milk, however, appear to accentuate compensatory villus growth after intestinal resection in suckling rats, and breast milk also appears to bring about further acceleration of mucosal maturation.

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AIMS OF THE PRESENT STUDY

1. To determine how well and how rapidly the residual intestine of breast fed and immature rats responds to the loss of absorptive surface area.
2. To determine factors, or nutritional manipulations, that might inhibit normal and regenerative growth of the residual small bowel after intestinal resection in young animals.
3. To determine if known biological mitogens, found in gastric secretions, saliva and in breast milk, would enhance the growth and adaptational response of the residual small bowel after intestinal resection.
4. To determine if the neonatal bowel is more or less permeable to antigenic macromolecules after intestinal resection.

INDICATIONS FOR THE EXPERIMENTAL STUDIES

Until the introduction of parenteral nutrition, neonates were assumed to be incapable of surviving even minimal intestinal resections (284). The introduction of effective nutritional support, however, has changed that situation entirely (90, 296, 345, 391, 392), and many neonates now survive massive intestinal resections of the small and the large bowel (203, 266). While Volvulus Neonatorum and other congenital defects (345, 379) continue to demand extensive resections of the gastrointestinal tract, the complications of neonatal Necrotizing Enterocolitis (N.E.C.) have become one of the most common reasons for extensive intestinal resections in the neonatal period (120, 203). N.E.C. is a disease process that is associated with prematurity, and as many more pre-matures now survive as a result of the opening of neonatal intensive care units, a significant number of these prematures live, only to develop N.E.C. (266, 280) which has been reported in up to 27% of those neonates born weighing less than 1200 grams (40, 265). As up to half of the neonates that develop N.E.C. have required intestinal resection (280), an increasing number of intestinal resections are being carried out in premature neonates (203). If these neonates are to survive and grow normally, then they must all be intensively and expensively supported (390) until such time as the residual intestine recovers from the original disease process (57) and also adapts to the loss of absorptive surface area (391). This adaptation is critical, as the intestine of both the premature and the full term neonate is working at, or near, its absorptive capacity even under normal circumstances (41).

Massive bowel resections in the neonatal period leave their legacy as a short fall in the physical and intellectual development in the early years of life (38, 344, 360, 361). Although the intellectual and the physical impairment may eventually disappear in the majority of cases (26, 38, 296, 360, 361), in others the defects remain (23, 296, 344). The development of impairment cannot be blamed on the surgical stress alone, as neonates that undergo major abdominal surgery without intestinal resections develop normally, whereas those that undergo small bowel resection as well

do not. The implication is that the loss of absorptive surface area and the subsequent interference with nutrition are responsible for the poor physical and intellectual development (344).

In the adult human being, when large areas of the gastrointestinal tract are removed, there is malabsorption, weight loss and diarrhoea (8, 25, 56, 57, 64, 70, 286, 314, 367, 381). With the passage of time, however, increasing adaptation to the loss of absorptive surface area occurs, and the nutritional loss and fluid losses gradually subside (8, 25, 56, 57, 64, 86, 295, 331, 381). In human neonates, clinical observations suggest that the long term response to massive intestinal resection may be more effective than the response to similar intestinal resections in the adult (295, 296, 318). The reasons for the more effective response, however, are not clear. Although there appears to be increased glucose absorption and sucrose hydrolysis per unit length of intestine after partial enterectomy in the newborn (319); improved segmental glucose absorption also occurs in the adult human being after intestinal resection (86).

During the period when adaptation is occurring, nutrition is supplied by vein. Nevertheless, the parenteral nutrition that is necessary to save the neonate's life has its dangers, as in adults (140) but, in addition, children, and especially premature babies, are prone to cholestasis (22, 24, 27), cholelithiasis (24) and liver fibrosis progressing to hepatic cirrhosis (191). Even if the neonate survives the nutritional problems associated with a massive intestinal resection, there is an increased risk of death from generalised infection (235, 377). One possible explanation for this may be the poor nutritional and immunological state (235, 340, 377), but another may be that the mucosal damage associated with the initial disease process, and the subsequent intestinal resection itself, may both combine to leave the intestine in a state of increased permeability to antigenic macromolecules (31, 75, 293, 294, 363). These macromolecules may be pathogenic viruses (394), endotoxins (166, 183, 273, 395) or food antigens that have the capacity to form immune complexes and cause local tissue dam-

age (82, 83). Moreover, human neonates, and especially premature neonates, have increased intestinal permeability to macromolecules (21). Furthermore, as compensatory intestinal regeneration in the residual small intestine involves mucosal hyperplasia (381), this hyperplasia itself may increase the number of sites at which macromolecules could cross the mucosal barrier (at the sub microfold cell, the M cells) and gain access to the circulation.

CHAPTER ONE

HISTORICAL REVIEW

In 1888, Senn observed that after intestinal resection in dogs, the wall of the residual intestine had become much thicker (323). Later, in 1896, Monari confirmed these findings in dogs, and also noted an increase in the numbers of villi in the residual small bowel (253). In 1912, Flint again demonstrated that, after intestinal resection in dogs, the mucosa became thicker, the villi increased in height, and also the intestine underwent dilatation so that the increase in the residual intestine's surface area was virtually 200% (107). Flint could not confirm the increase in the numbers of villi, however, at variance with Monari's findings, nor could he record any elongation of the residual bowel. Furthermore, Flint noted that the younger dogs failed to tolerate extensive small bowel resections, as well as the older dogs, and in 1942, Petri confirmed this relative intolerance to intestinal resections by demonstrating growth failure and death in young dogs after distal intestinal resections (279).

By 1952, however, Clatworthy had shown that the suckling puppy could tolerate up to an 88% small bowel resection without growth failure, but again he noted the lack of compensatory elongation of the residual intestine when its growth was compared with the normal elongation that had taken place in the intestine of the control

animals (70). When massive intestinal resections were performed on neonatal pigs and mini-pigs, failure to thrive and death were often noted (17, 237, 295), but in some of the animals that survived compensatory elongation of the residual bowel was observed (295). When 90% were carried out in puppies, to the extent that they required parenteral nutrition to survive (391), then intestinal elongation over and above normal growth was noted in the intestine of survivors. Furthermore, when a 90% intestinal resection was carried out in more mature rats, there was marked compensatory elongation of the residual intestine within one week (307).

As recently as 1955, Potts stated that the human neonate could not survive if more than 15% of the small intestine was removed (284), but by 1967 Benson had reported survival of a human neonate when only 12% of the small intestine remained (26). In the same year, Rickham stated that the power of compensatory regeneration after intestinal resection in the neonate was far greater than that found in adults or in the older child, and that intestinal resection in the neonatal period was becoming commonplace (323). By 1978, survival and independent existence without the need for long-term parenteral nutrition was reported by Tepas when only 15 cms (or 7%) of the small bowel remained (314), and independent survival with as little as 12 cms of small bowel has been recorded after the long-term use of parenteral nutrition combined with feeding breast milk (128).

LITERATURE REVIEW

1. IMPROVED COMPENSATORY REGENERATION IN YOUNG ANIMALS

After partial hepatectomy or unilateral nephrectomy, compensatory regeneration is more marked in the suckling animal than it is in the mature animal (19, 51, 52, 53, 236, 240), although more recent studies adopting an allometric approach throw some doubt on the observation that compensatory renal growth is more marked in

the young mouse (163). What is more, the regenerative capacity for both renal and hepatic tissues decreases with advancing age (19, 51, 53, 240). Compensatory regeneration of the intestine (adaptation) is also well documented after partial enterectomy (381) but, although increased villus height, intestinal elongation and intestinal dilatation have all been documented after intestinal resection in the human neonate (23, 252, 295, 319, 182), these observations and animal experiments have failed to establish clearly whether the intestine of the human neonate can regenerate after partial excision more effectively than the intestine of the adult.

Adaptation is the regenerative response to intestinal resection. The response to resection of the small bowel consists of structural, functional and cyto-kinetic changes that, on balance, improve the absorptive capacity of the residual bowel. There are, however, several differences between the morphological, kinetic and functional states of the intestine in the neonatal and the adult mammal that would suggest that the adaptational response would be different in the two.

2. STRUCTURAL CHANGES AFTER INTESTINAL RESECTION

2.1 Normal Structure, Adult and Neonatal

In normal neonatal animals, the intestine has more length in relation to body weight than at any later stage in its development (59). In the human, intestinal length in the prematurely born may be as little as 150 cms, whereas in the full term the intestine measures approximately 250 cms (26, 313). Obviously, therefore, the potential exists for the residual intestine to grow more after intestinal resection in the premature, than later on in development and in later childhood, when intestinal growth gradually declines, as normal intestinal elongation ceases at approximately 5 years of age (48, 295). Intestinal growth is not limited to intestinal length alone, as the muscle and mucosal layers are also growing in the neonatal period (59). In the human neonate, for

example, the bowel wall is noticeably thin because of this lack of muscle (59).

The mucosal morphology of the developing intestine is quite different from that of the adult animal. In the neonatal rat, for example, the villi mature just before birth, and then gradually grow throughout the suckling, weanling and post weaning periods (11, 151, 351). The crypts, however, undergo an abrupt growth spurt as weaning commences (151), and at the same time the cell population in the intestinal mucosa increases and the individual cells grow in size (201). The changes that occur during mucosal maturation are not immutable, as they can be influenced by exogenous stimuli in the experimental animal. For example, mucosal maturation can be accelerated by corticosteroids and thyroxin, and may be influenced by gastrin (151, 222).

In the human foetus, changes in mucosal growth occur much earlier than in the rat, as essentially mature villi are present by the mid-trimester of pregnancy (351), but gradual growth of the villi continues until term. Crypt growth, on the other hand, is slow during the first and second trimesters, accelerating in the last trimester, so that the crypt depth to villus height ratio changes abruptly in the late stages of pregnancy (213), a similar pattern to the changes in crypt depth that occur in the intestine of the suckling rat at the time of weaning.

At the cellular level, development follows a similar pattern in the human foetus and in the suckling rat, but again changes occur earlier in the human. The enterocyte of the 10-20 week human foetus resembles that of the suckling rat (351), and the meconium corpuscles, most prominent in the ileum of the 22-week human foetus, may well represent the supranuclear vacuolation in the suckling rat which plays an important role in the absorption of intact macromolecules (339).

2.2 Macroscopic Changes

The macroscopic changes that occur after intestinal resection are: thickening of the bowel wall (hypertrophy) (42), dilatation and elongation (42, 271, 307), and an increased blood supply (349).

2.2.1 Hypertrophy of the bowel wall

After partial enterectomy in the adult animal, all the coats of the bowel wall hypertrophy, but the mucosa contributes more than the muscle coat (42, 87, 107, 135, 136, 269, 271). The changes in the mucosa precede those in the muscle (267). This hypertrophy of the mucosa and of the muscle coat is not merely the result of distal obstruction, as the hypertrophy is more marked distal to the anastomosis than it is proximally (107, 267, 269). The neonatal animal is also capable of adaptation as, after intestinal resection in the neonatal minipig, hypertrophy of the mucosa of the residual intestine has been reported after the animal had virtually grown to adult size (17, 295). In humans, hypertrophy of the bowel wall has also been observed after intestinal resection in the newborn and adult (23, 162, 182, 314, 319).

2.2.2 Dilatation of the residual intestine

A constant response to intestinal resection in adult animals is dilatation of the residual intestine (42, 269, 271, 307, 381). The response, like the muscle wall and mucosal thickening, is not merely the result of obstruction and dilatation of the intestine, as passive dilatation is accompanied by thinning of the bowel wall, and not hypertrophy (42, 269, 271). Furthermore, the dilatation is again more pronounced distally than it is proximally (269). Compensatory dilatation, as well as increasing the absorptive surface area of the residual intestine, may play an important role in slowing of the intestinal transit time of nutrients after intestinal resection (269). As a result, there would be more time available for intraluminal digestion to occur (381). Compensatory dilatation of the intestinal tract after intestinal resection has also been documented in the neonatal animal (17) and in newborn and adult human beings (23, 182, 252, 314).

2.2.3 Elongation of the intestine

Elongation of the residual intestine might be expected to bring about marked improvement in function by increasing the absorptive surface area and increasing transit time. At the same time, intestinal elongation could not detract from the other structural aspects of intestinal adaptation. Unfortunately, however, intestinal elongation is an inconsistent response to intestinal resection in adult animals and responses are not observed until particularly extensive resections have been performed. Even then, there appears to be a species difference. In early studies on adult dogs, resections were less than critical (107, 253, 323), and in those studies elongation of the residual bowel did not occur even if 90% of the small bowel was removed (107).

When up to 80% intestinal resection was performed in rapidly growing suckling puppies, the residual intestine again failed to elongate more than would normally have been expected for that segment of bowel at that age (70). But, when 90% and 95% small bowel resections were carried out in weaned puppies, the residual intestine of those puppies that were supported by both parenteral and enteral nutrition demonstrated approximately 35% greater than normal elongation of the residual intestine one year later. In those puppies that died after the same degree of intestinal resection, and in whom only enteral feeding had been employed, there was no such compensatory intestinal elongation and, interestingly, there was not mucosal hypertrophy either (391).

After intestinal resection in the neonatal minipig, variable intestinal responses were recorded, and again the response apparently depended on the nutritional state of the animal, as well as the length of bowel removed (295). In yet a different species, the rat, less extensive intestinal resections produce elongation of the residual intestine. But, again, the response depends on the extent of the gut removed. For example, a 75% small bowel resection will produce compensatory elongation within 7 months

(269), and 90% small bowel resection will produce compensatory elongation within a week (307). In summary, therefore, there appears to be a variation in compensatory elongation of the intestine after intestinal resection that not only depends on the species involved, but also depends on the length of the intestine removed, the post operative time interval, and the nutritional status of the animal (307, 381, 391).

Although compensatory elongation of the small intestine can occur after particularly extensive resections in the adult human being (314), intestinal elongation over and above normal growth is difficult to define in the child (295). Nevertheless, after intestinal resection in one human neonate, almost 100% intestinal elongation occurred within 12 months (252), and approximately 260% elongation after 18 months in another child (295). Furthermore, out of a series of eight children undergoing re-operation after neonatal intestinal resection, up to 450% intestinal elongation was recorded (182). Those undergoing the most extensive resections demonstrated the greatest elongation, suggesting that the resection was acting as an added stimulus for the intestine to elongate more than normal (182).

2.2.4 Vascular changes

An increase in the vascularity of the residual intestine was first noticed by Senn in 1888 (323), and more recently it has been suggested that the adaptational response to intestinal resection may be initiated by the increased blood supply to the residual small bowel (349). This increased blood supply is more marked in the distal small bowel - that part of the intestine that shows the most marked adaptational response (381). But, although the changes in the blood supply precede the changes in the villus height, they do not precede the almost immediate increase in DNA synthesis that occurs in the crypts after intestinal resection (346, 349).

2.3 Microscopic Changes After Intestinal Resection

The microscopic changes that occur after small intestinal resection include changes in the size and the shape of the villi, possibly increases in the number of the villi, changes in crypt size, the number of mitoses per crypt, and possibly the number of crypts in the residual small bowel.

2.3.1 Villus Height

After intestinal resection in the adult rat and dog, the villi elongate within 7 days, reaching a maximum response within 12 days (381). The major part of this response is achieved by a massive increase in the number of cells per villus (hyperplasia), but not by an increase in the individual cell size (hypertrophy) (381). Nevertheless, there is conflicting evidence as to whether or not cellular hypertrophy, or even hypotrophy, occurs after intestinal resection. When the RNA:DNA ratios are used as an indicator of cell size, the enterocyte size may actually decrease after intestinal resection in the adult rat (376). Other studies, however, showed either no change in cell size (388), or even an increase in cell size (324), after intestinal resection, when RNA:DNA ratios were recorded.

When intestinal resections were carried out in the neonatal minipig, the suckling, or the weaned puppy, the changes observed in villus height were similar to those seen in the adult animal (17, 70, 295, 391), not surprisingly, when sacrifice was carried out well after suckling had ceased. As a result, the short-term response of the villus within 7 days of an intestinal resection, and purely within the suckling period, is largely unknown.

After intestinal resection in one human neonate, duodenal biopsy 12 months later demonstrated increased villus height in comparison with a control biopsy (23). In a series of 5 children biopsied after intestinal resection, there was a 66% increase in villus height in comparison with controls (319). After intestinal resection in the adult human being, however, increase in villus height

has yet to be recorded, although an increase in the number of cells per unit length of the villus has been observed (162, 283, 381). Even then, this increase in cell number is only observed after more than 75% of the small intestine has been removed (283, 371). This increase in the cell numbers per unit length of the villus would tend to support the initial suggestion that the enterocytes decrease in size after intestinal resection (376). Nevertheless, increases in villus height have been recorded in the in-line intestine after jejuno-ideal bypass in the human (see Section 3.1.1).

2.3.2 Villus shape

When proximal intestinal resection is performed in adult animals, the normally slender villi of the ileum change shape from finger-like processes to leaf-shaped projections similar to normal jejunal villi. As a result, the absorptive surface area increases (100, 327). The villus of the human neonate apparently has the added capacity to produce branched villi after an intestinal resection (23).

2.3.3 Villus numbers

The evidence for or against increase in the numbers of villi in the residual intestine after intestinal resection is also conflicting. The early work of Monari suggested that there was an increase in the numbers of villi after intestinal resection in the dog (253), and this finding was later re-affirmed for the dog (327) and for the rat (36, 269). Other investigators have, however, thrown doubt upon these findings (107), and one careful morphometric study of the whole of the residual intestine of the rat failed to show any increase in villus numbers after intestinal resection, although there was extreme individual variation (110). As functional improvement continues long after the villi have reached their maximum height (8, 23, 26, 56, 86, 296, 345, 381) at 6 to 12 days after the intestinal resection (381), it is tempting to speculate that, as the villi increase in numbers to cover the increased circumference of the gut (269), this increase in the

villus population is the reason for the continuing functional improvement. Nevertheless, the slowing of the intestinal transit time (267, 271), and the increase in the breadth of villi (137), could both contribute to the prolonged functional improvement.

2.3.4 Crypt responses

The crypt column (the number of cells on one side of a crypt) elongates within 4 days of an intestinal resection (136). By 12 days after an intestinal resection, the crypt size has reached its maximum, and no further increases are seen at 30 or at 60 days after partial enterectomy (136, 240). Evidence for an increase in crypt numbers after intestinal resection has not been found (135) but, after extensive jejunal bypass, increased crypt:villus ratios have been recorded in that part of the intestine that remains in the nutrient stream (111). That part of the intestine undergoes similar changes to the residual intestine after partial enterectomy (381).

2.4 Ultrastructural Changes

Changes in the size of the brush border villi have been recorded after intestinal resection but, just like many of the other parameters of intestinal adaptation, there is no universal agreement as to which direction that response takes. Two reports suggest that there is no change (125, 391), and yet another report suggests that there is even a decrease in the size of the microvilli (118).

3. STRUCTURAL CHANGES AFTER OTHER PROCEDURES

3.1 Intestinal Bypass

When part of the intestinal tract is bypassed so that it is removed from the nutrient stream, the opportunity exists to determine the separate effects of luminal or systemic influences that might be involved in the mucosal response to the loss of absorptive surface area.

3.1.1 The mucosal response to bypass

The intestine that remains in the nutrient stream after intestinal bypass undergoes an adaptational response similar to that seen in the residual small intestine after partial resection (121, 249, 269, 307) but, although no increases in the numbers of crypts can be detected after intestinal resection (135), the crypt:villus ratio increases in the in-line intestine after bypass (68).

The bypassed segment behaves in a different manner. There are progressive decreases in the number of cells per crypt, a contraction of the crypt size, a decrease in the villus height, and a narrowing of the villi (249, 297), although occasional reports suggest that there is no atrophy (246). The influence of luminal nutrition may be further inferred when the jejunum (that part of the intestine normally bathed in a high concentration of nutrients and pancreatico-biliary secretions) is seen to atrophy more than the ileum (that part of the bowel not normally bathed in high concentrations of chyme) when either one is bypassed separately (137). The atrophic response after bypass, however, is not simply the result of luminal nutrition, as the degree of mucosal atrophy is not complete under all circumstances. When more of the in-line intestine is removed, there is less mucosal atrophy in the bypassed segment, suggesting that the residual in-line intestine in some way stimulates the bowel that has been bypassed. This could be an hormonal enterotropic factor, or a reflex neurological stimulus (92, 137, 206, 347, 354, 355, 383, 389). Further evidence for an enterotropic factor stems from observations that, when part of the intestine of otherwise normal lactating rats is bypassed, the by-

passed segment also shares in the generalized mucosal hyperplasia associated with lactation (60).

The intestine in humans responds in a similar way to that of the experimental animal. In the adult human being, although increases in the villus height have not been reported after intestinal resection, increases in the villus height have been reported after jejunio-ileal bypass for morbid obesity, where again it is the in-line intestine that undergoes the adaptational response to the loss of absorptive surface area (89, 103, 329).

3.1.2 Macroscopic responses

The mucosa is not the only part of the intestine that takes part in the structural changes after intestinal bypass. In the adult rat, there is an increase in the muscle mass, which is observed some time after the mucosal changes have taken place, and which may be the result of the increased bulk of nutrients reaching the distal bowel (267).

In growing animals, where intestinal bypass has been carried out in the rabbit or rat, the bypassed segment does not grow normally, and may even decrease in length (190, 269). Furthermore, when bypass has been performed in neonatal piglets, the bypassed segment atrophies, although total gut length is normal (238). There is no data available for intestinal bypass in the human neonate.

3.2 Parenteral Nutrition

Parenteral nutrition abolishes the need for any nutrients to be present in any part of the gastrointestinal tract, and at the same time it avoids starvation. Thus, both the direct stimulus of luminal nutrition, and the indirect stimulus of luminal nutrition elsewhere in the gut may be avoided (92, 137, 206, 347, 354, 355).

3.2.1 Parenteral nutrition in normal animals

In normal animals, parenteral nutrition produces marked reduction in mucosal villus height, protein and DNA content. These changes are most marked in the jejunum (174, 216, 267), and are present within three days (159). Interestingly, when rats are fed on an elemental diet, similar, but less marked, changes are observed (96). In response to parenteral nutrition, the microvilli do not change in height (61). In the normal immature rat, parenteral nutrition inhibits mucosal growth (257), and in the 8-10 week old puppy, parenteral nutrition inhibits both mucosal and longitudinal growth (202). Therefore, parenteral nutrition apparently has the potential to suppress normal intestinal growth.

3.2.2 Parenteral nutrition after partial enterectomy and bypass

Parenteral nutrition also reduces the response to intestinal resection in growing rats (258), and may even abolish the response altogether in the more mature animal (101). Furthermore, when rats are placed on parenteral nutrition, and part of the intestine is bypassed, the mucosa in the bypassed segment has less DNA content than similar loops in normally fed animals, despite some residual degree of hyperplasia being present in the in-line segment (104). This suggests that, under these circumstances, the systemic effects of luminal nutrition are completely absent, and that these reflexes normally come from the stimulated in-line segment. Possibly, the residual hyperplasia in the in-line intestine is the result of basal secretions entering the upper gastrointestinal tract.

In man, when adults are placed on parenteral nutrition and luminal nutrition after massive intestinal resection, mucosal hyperplasia does occur, as does minimal intestinal elongation (314). But, when human neonates are supported by parenteral nutrition after massive intestinal resections, functional adaptation may take up to 6 months to occur (126). While this could be the time taken for sufficient normal gut growth to occur to enable adequate absorption (295), the parenteral nutrition may, in itself,

have prolonged the recovery phase, by inhibiting the adaptational response, and possibly even normal gut growth as well. In support of this suggestion is the observation that, when premature neonates are placed on parenteral nutrition, the one hour xylose absorption test demonstrates reduced uptake of xylose in comparison with similar, but enterally fed, premature babies (162).

3.3 Starvation

When adult rats are starved, the villi and crypts decrease in size, cell proliferation slows (4, 10) and, although villus numbers do not change, the crypt numbers may decrease (65). When suckling rats are starved, the villi fail to grow, the crypts diminish in size as cell proliferation is reduced, and enterocyte maturation is delayed (127, 139, 277). When children suffer from chronic malnutrition, villus atrophy is also found (138).

4. KINETIC CHANGES AFTER INTESTINAL RESECTION AND OTHER PROCEDURES

4.1 Normal Cell Kinetics, Adult and Neonatal

In the mammalian small intestine, the crypts of Lieberkuhn are the only site of enterocyte production. Cell division is further restricted to the base of the crypt where DNA synthesis is rapid, and where the enzymes involved with DNA synthesis are found (164). Once the cells leave the lower crypt zone, thymidine kinase activity disappears (94, 226), and division is no longer possible as the cells lose the capacity to incorporate thymidine into DNA.

Cell turnover time (the time it takes for the enterocyte to be produced, migrate along the crypt and the villus, and be extruded), is accomplished within 2-3 days in the adult mouse and rat, and within 3-6 days in the adult human being (381). Cell migration from the crypt to the villus tip is accomplished by movement of cells out of the crypt, as newly formed cells take their place. The enterocytes that line the villi then migrate towards

the villus tip, accompanied by the underlying mesenchyme, so that this whole intestinal "proliferon" (401) is constantly renewed at the level of the crypt base, and extruded at the level of the villus tip. The cell turnover time in the jejunum is more prolonged than that in the ileum, as the villi in the jejunum are taller, and migration rates (the rates at which the cells travel along the villus), are the same (11). In the adult rat and mouse, there is a circadian rhythm of DNA synthesis throughout the whole of the gastrointestinal tract (315, 316, 354) and, possibly, this rhythm is governed by food intake (316). In the suckling rat, no such rhythm exists, because the mother imposes daytime suckling on her pups (147).

Information as to crypt cell kinetics in the human being is minimal, but what little exists would suggest that crypt cell production in the human neonate is slow at birth, and accelerates later in life (152), but these observations were necessarily carried out on dying neonates. In the experimental neonatal or suckling rat, crypt cell production is slow, but abruptly increases tenfold at weaning (11). As a corollary, cell turnover time reduces from 6-10 days, to 2-3 days, as cell migration rates increase (202, 339) as the rat matures from suckling to independence. This new cell production rate continues until old age (69). In the senile rat, there are reports of increased villus numbers, crypt numbers, and a constant cell production rate per crypt (97); or the opposite, with a small reduction in villus height and the total number of villi (69, 110), and also an increase in crypt cell production (76).

As the structural changes in the mucosa can be influenced at the time of weaning by exogenous influences, so the normal cyto-kinetic changes in the maturation process can be accelerated by steroids given in pharmacological and physiological doses (150, 151, 200, 201), and crypt cell kinetics can also be influenced by neural stimuli (206, 354, 355).

4.2 Cyto-kinetics After Intestinal Resection

4.2.1 Cell proliferation

Nearly all of the observations made on cell kinetics after intestinal resection have been made in adult animal models. Within 24 hours of an intestinal resection in the adult rat, before any increase in the villus height is observed, DNA synthesis within the crypt increases (346). This early change brings about an increase in the number of cells within a crypt column that will take up tritiated thymidine. As the crypt column size does not increase at this early stage, the labelling index (that portion of the crypt column that contains cells that take up isotope, expressed as a fraction of the whole crypt column) increases (346). Once newly formed cells have moved up into the upper part of the crypt, however, the crypt elongates, and the labelling index returns to normal (135, 136), as a new steady state of increased cell output is achieved.

Despite the already rapid rate of cell proliferation in the normal adult mammal, increased cell production can, and does, occur after intestinal resection (381). There is intense epithelial regeneration with increases in DNA and RNA content of the residual mucosa (136, 272, 384, 388, 391), which occurs within 24 hours of an intestinal resection in both the colon and the small intestine in the rat (136, 272, 384, 388). To accomplish this acceleration in cell reproduction, there is a shortening of the cell cycle time, with increased activity of the enzymes associated with DNA synthesis (263, 381).

4.2.2 Cell migration

Disagreement exists as to the rate of cell migration after intestinal resection. It has been reported to remain the same as before resection (134), or to increase after intestinal resection and bypass (88, 121). As a result, the cell turnover time (which is dependent on the rate of cell production, cell migration and cell extrusion) has been said to increase as the villus elongates

(134, 136), or to stay the same, as both the cell migration rate and the villus size can change independently (88, 121).

Although luminal nutrition plays a central role in the villus response to intestinal resection (45), luminal nutrition cannot be responsible for the early proliferative changes that are seen in the crypt while the animal is being fasted immediately after surgery (346).

4.3 Altered Cell Kinetics After Intestinal Bypass

When small bowel is excluded from the intestinal stream, the bypassed bowel retains its blood borne nutrition and its innervation. Essentially, the changes that take place in the residual in-line intestine are the same as those that take place after enterectomy, but the initial rate of response may be somewhat slower than after intestinal resection (381). Cell proliferation within the bypassed segment falls but, despite the decrease in the villus size and the crypt depth, the time taken for cell turnover actually increases as the rate of cell migration from crypt to villus tip slows disproportionately (67, 297). Because the rate of cell proliferation slows as the crypt column diminishes in depth, the labelling index and the tritiated thymidine labelled DNA per mg of total DNA remains unchanged (297, 381).

While it is probable that the reduced luminal nutrition is the major factor responsible for the reduction in mucosal mass, luminal nutrition cannot be responsible for the increased crypt cell proliferation in the bypassed segment of bowel after bypass of particularly extensive lengths of small bowel, when compared with the rate of crypt cell proliferation in bypassed segments when only short segments are removed from the intestinal stream (2, 92, 137). This suggests that the systemic stimulus released from the residual in-line intestine is proportional to the amount of intestine taken out of the luminal stream (137, 383, 389).

4.4 Altered Cell Kinetics and Parenteral Nutrition

When rats are fed intravenously, DNA synthesis in the small intestine diminishes (306), as the DNA content falls (92, 159, 174). Similar falls in the DNA content are observed when immature rats are fed intravenously (257). Falls in mitotic index were also seen when puppies were placed on parenteral nutrition (202).

4.5 Altered Cell Kinetics After Starvation

When the intestine is denied both luminal and blood borne nutrition, there is a disproportionate atrophy of the intestinal tract when compared with other parts of the body (337). In the adult rat and mouse crypt, as after bypass and parenteral nutrition, cell proliferation again diminishes, with prolongation of the cell cycle time (4, 10). Cell migration slows, and decreased renewal of crypt cells occurs in either total starvation or with protein deprivation alone (158, 197, 228). At the same time, the DNA content of the intestine falls, and the actual number of crypts may reduce (65).

In the suckling rat subjected to malnutrition, crypt and villus size diminish with corresponding decreases in DNA synthesis and decreases in the cell migration rate (127, 139). These changes, and the changes seen after parenteral nutrition and bypass, are completely reversible (4, 10).

5. FUNCTIONAL CHANGES AFTER INTESTINAL RESECTION AND OTHER PROCEDURES

5.1 Normal Gastrointestinal Development

The functions of the gastrointestinal tract are different in the adult and neonatal animals. As the development of the gastrointestinal tract of the laboratory rat has been most studied, the major part of this section will concentrate on the developing intestine of the suckling rat. At the time that it appears to be

structurally immature (351), and at the time that cell proliferation and cell migration are at their slowest (202, 260, 339, 351), the intestine of the suckling rat is, in fact, well adapted to the digestion and assimilation of breast milk (147, 255). The enzymes necessary for this function are present throughout the suckling period, and those necessary for an independent omnivorous existence appear later, as weaning progresses (13, 133, 147, 179, 199, 204, 255, 281, 335, 339, 359). The function of the intestine at any age, however, is to absorb carbohydrate, fat and protein.

5.1.1 Development of carbohydrate absorption

Rat breast milk contains lactose and neuraminlactose as the only carbohydrate sources. These increase in concentration gradually through the suckling period (123, 204). In the suckling animal, however, luminal hydrolysis is limited by pancreatic immaturity (147). Therefore, to digest these disaccharides, mucosal lactase activity is high at birth, and remains high until the rat starts to wean onto other carbohydrates (147). At that time, the lactase activity starts to drop, and reaches the normally low adult values at approximately 20-25 days of age (147). Intestinal brush border sucrase activity, one of the most reliable, and one of the most often assayed markers of intestinal functional maturation (147), is undetectable at birth, and until 15 days of age; then, at 17 days of age, sucrase activity abruptly increases to reach a maximum at approximately 24 days of age, as the rat weans, and sucrose becomes one of the major sources of dietary carbohydrate. Maltase activity follows an essentially similar pattern to that of the development of sucrase activity (147, 199, 204, 255).

As well as altering the structural and cytokinetic changes, exogenous stimuli can alter the functional changes associated with mucosal maturation also. The normal pattern of functional development can be influenced by surgical and nutritional stress, and by the actions of hormones. Corticosteroids have been shown to change the pattern of mucosal disaccharidase development (150, 180, 188, 200, 210, 255, 339). Sucrase activity appears early after injections of large and physiological doses of corticosteroids and acid

β -galactosidase activity falls (200). Furthermore, adrenalectomy delays these changes (150, 180, 188, 200, 210, 255, 339). Thyroxine, however, also plays a central role, and appears to be the major hormonal stimulus for the fall in lactase activity, as injections of thyroxine can accelerate this fall in lactase activity (181), and thyroidectomy or hypophysectomy delays it (84, 180, 200, 255, 399). Nevertheless, the two hormones are not totally independent of each other in their effects on disaccharidase activities, as both of them will affect, to some degree, both lactase and sucrase activity (180, 255, 399), and the developmental pattern of either enzyme activity is not completely normal if one of the hormones is absent (84, 181, 255).

Although the change in diet coincides extremely well with the changes in enzyme activity seen throughout the gut, the changes in activity are not merely the result of the dietary stimulus. If rats are prevented from weaning, but continue to suckle, then these changes still occur, although the time sequence may be altered, and the fall in lactase activity is delayed (210). Not only that, but when foetal rat tissue is placed under the renal capsule of a syngeneic adult rat, the intestine goes through somewhat altered, but essentially normal, maturation patterns, even when it is away from the influence of food, and the hormonal status of the host is stable (188). Therefore, there must be an inherent time clock present in the cells of the intestine, that is capable of switching on the changes in enzyme activity that normally occur at weaning (81, 187, 199, 254), and can do so wherever the gut may be (105). This time clock also appears to influence the circadian rhythm of enzyme activity that develops in the older rat, as such a rhythm cannot be imposed in the suckling rat by altering the feeding patterns (311). Furthermore, this time clock may not be the appearance of the changes in enzyme activity itself, but could well be a change in the sensitivity of the enterocyte to normal levels of circulating hormones, so that, although normal levels of circulating hormones could not induce the enzyme changes early on, the same concentration of the same hormone can do so as the cell's sensitivity increases (150, 200). In this way, stress should switch on the

appearance of sucrase activity as normal weaning approaches, with minimal changes in corticosteroid levels. Even so, at the time of normal weaning, there is an abrupt rise in corticosterone levels in the rat (146) that could well promote the normal rapid changes in enzyme activity.

In the human foetus, the developmental patterns of disaccharidase activity are somewhat different from that in the rat. Sucrase activity, for example, is present at 10 weeks of gestation, and reaches peak values at 34 weeks. On the other hand, although the appearance of lactase activity is earlier in the human foetus, the overall developmental patterns are similar in the rat and the human being. In the human foetus, lactase activity can be detected at 10-12 weeks of gestation, and reaches a peak as late as 40 weeks gestation (13, 14, 199, 204, 255, 359). As in the rat, stress will induce changes in the disaccharidase activity but, in the human, lactase appears to be the more sensitive enzyme system. After surgical and other stresses, lactase activity can disappear early, leading to lactose intolerance (152). Not only that but, after surgery and diarrhoeal illnesses, even monosaccharidase intolerance can occur (3, 55).

5.1.2 Development of fat digestion

In the neonatal animal, pancreatic and hepatic function are immature, so that intraluminal fat digestion relies heavily on gastric lipolysis. It is probable that this gastric lipolytic activity is partly derived from lingual lipase swallowed with breast milk (133). In the presence of lingual lipase activity, fat digestion can take place, even in the absence of bile and pancreatic juices (124), and the triglycerides in native milk that are resistant to pancreatic lipases are readily hydrolysed in this way (72). Even in the presence of bile and pancreatic juices, triglyceride hydrolysis is accelerated by pre-incubation with lingual lipase (133), and exclusion of saliva from the stomach impairs fat digestion in suckling rats (281).

In the human neonate, lipase activity is present in the stomach at birth, and the blind pouch of an oesophageal atresia (133). That this activity is important to the human neonate is demonstrated by the increased faecal excretion of fat if human neonates are fed naso-jejurally, as opposed to feeding them naso-gastrically (304). Furthermore, in the premature neonate, pancreatic lipase activity, and bile acid secretion, are both reduced. Pancreatic lipase activity at 32-34 weeks gestation is only 50% of that at term (403); bile acid concentration at 32-34 weeks of gestation is only 50% of that at term, and only 25% of that in the adult (369, 370).

Despite the improvements in fat digestion brought about by the action of lingual lipase, fat digestion is incomplete in the suckling animal, and still continues well down into the small intestine of the suckling rat. As a result, there is distal dislocation of the other enzymes involved in digestion, especially the disaccharidases (359).

Other sources of lipase activity exist for the suckling animal. Primate breast milk contains a lipase that is activated by bile acids, and milk lipases have been shown to enhance fat digestion in the newborn human being (133). Mucosal lipase activity ("acid lipase") is present in high concentrations in suckling rats (71), and aids in breast milk digestion. Mucosal lipase activity, unlike mucosal disaccharidase activity, does not appear to have an inherent clock for its induction, and its activity climbs in response to breast milk feeding (326).

Despite the low pancreatic lipase activity, the low bile salt production, and the small bile salt pool, fat digestion is moderately effective in the human neonate, despite some losses in the stool (368). Lingual lipase digests fat early, and the products of early digestion, fatty acids and monoglycerides, compensate for the lack of bile salt by emulsifying the lipid mixture. Furthermore, one potential mechanism whereby fat can be absorbed in the neonate may be as intact macromolecules (186).

As can be seen from these observations, fat digestion is precarious in the suckling animal and the human neonate, and when intestinal resection reduces the absorptive surface area further, steatorrhea is a frequent complication (295, 296, 360). Even so, the older child who has been subjected to an intestinal resection in the neonatal period copes extremely well with this fat malabsorption (295, 296, 360).

5.1.3 Development of protein digestion

Although the human newborn has some capacity to digest proteins at birth, that function is not fully developed (192). The gastric phase of protein digestion depends on pepsin and, although pepsin has been demonstrated in the 16 week human fetus, term infants only have 10% of adult values of pepsinogen content in their chief cells (372). The intraluminal phase of protein digestion depends on the presence of pancreatic trypsinogen, chymotrypsinogen, proelastase, and procarboxypeptidases (192). Enteropeptidase (enterokinase) from the cells of the small intestine activates these pancreatic enzymes directly, and indirectly by activating trypsin. Pancreatic secretory granules have been observed at 5 months gestation in the human fetus, and tryptic activity can be detected in the pancreas by 4 months of gestation (74, 185). Although these pancreatic enzyme activities are detected early, proteolytic activity is not detected in the faeces until later, when the fetus has reached 32 weeks gestation (223). Enterokinase activity is apparently insufficient to activate the pancreatic enzymes, is only 6% of adult values at 20-30 weeks gestation, and has only reached 20% of adult values at term (14).

The third phase of protein digestion, the mucosal phase, has two mechanisms whereby the products of protein digestion can be absorbed. One is by absorbing the final di- and tri-peptides, and the other is by directly absorbing macromolecules. Varying levels of dipeptidase activity and aminopeptidase activity have been measured in the second and third months of gestation in the human fetus (153, 225, 305), and active transport mechanisms are present by the fifteenth week of gestation (215). However, as already

noted, enteropeptidase activity at birth is low compared with that found in older children and adults (14).

Macromolecular protein transport, in man, is of questionable significance *in utero* and after birth (111), but macromolecular transport remains as a potential mechanism whereby antigenic macromolecules may gain access to the systemic circulation. As a result, the human neonate is capable of transporting intact gamma-globulin antibodies across the intestinal mucosa (212a, 165). Furthermore, antigenic material can gain access to the immune system without necessarily gaining access to the general circulation, as antibody levels rise after an enteric challenge with common antigens such as bovine lactalbumin and bovine serum albumin (111, 227, 303).

As in the human neonate, protein digestion in the neonatal animal is not as effective as in the mature animal (147), because the gastric and the pancreatic phases of protein digestion are also immature. As a result, the rat relies heavily on mucosal peptidases to carry out protein digestion in the suckling period, and has a more marked capacity to transport intact macromolecules across the mucosal barrier (147). The adult pattern of pancreatic and gastric protein digestion appears at weaning (106, 299).

5.2 Intestinal Resection

After massive intestinal resection, absorption of nutrients, fluid and electrolytes is poor, so that major gastrointestinal losses occur. With time, however, fluid losses diminish, and the malabsorption diminishes (295, 296, 360, 381). This change is associated with an improved functional capacity of the residual intestinal tract (162, 318, 381). Transport studies have shown that absorption of simple nutrients per unit length of the residual intestine increases as mucosal hypertrophy occurs and, for glucose at least, the rate of absorption is essentially proportional to the villus height (249, 376). This increased absorption is not accomplished by an increase in the functional capacity of

each cell, however, as enzyme activity per mg protein does not increase in the residual intestine (162), and may even decrease after intestinal resection (376), especially for aminopetidases (100). Therefore, it is the vastly increased enterocyte cell population that accounts for the increased absorption, and not an increased absorptive capacity per cell (381, 376).

Neonates, animal or human, are especially vulnerable to the adverse effects of intestinal resection for, not only do they not have large energy stores (141, 169), not only do they have to absorb food for day to day needs as does the adult, but they also have to absorb nutrients in excess of that to grow (295, 296). At the same time, the intestine of the premature, and even the full term human neonate, is already working at, or near, its capacity to absorb both carbohydrate (40) and fat (108). After intestinal resection in human neonates, however, there is an increased segmental absorption of glucose and increased segmental sucrose hydrolysis (318).

5.3 Intestinal Bypass

After intestinal bypass, the in-line intestine undergoes functional changes that are similar to the changes seen in the residual small intestine after intestinal resection (381).

The in-line intestine adapts to the intestinal hurry that results from the shortening of the bowel (267, 271), but it will only do so if there is food present in the gastrointestinal tract (267). In the rat, not only does the mucosal response of the in-line intestine depend on the presence of food, but also the type of food. If a high protein diet is fed to rats after bypass, there is a marked increment in villus height, but little increase in water sodium and glucose absorption. If chow is given, however, there are marked increments in water, sodium and glucose absorption, but little increment in the villus height (246).

The bypassed segment not only undergoes structural atrophy, but function also falls away. In rats, glucose and tryptophan absorption are reduced in the bypassed segment of intestine (56, 85, 121, 249), and in the bypassed segment, enzyme activities per centimeter are reduced as the enterocyte population falls. Enzyme activities per mg protein or per mg DNA do not change, however (122, 297), demonstrating that the enzyme activity in each cell remains constant.

Little is known about the effects of intestinal bypass in the young animal, or the young human, but when intestinal bypass was carried out in the two week old piglet, the lactase specific activity in the bypassed intestine remained high, and other enzyme activities did not increase to their normal values (239). When intestinal bypass is carried out for morbid obesity in the adult human, one of the reasons for failure is the adaptation that occurs in the residual in-line intestine (89). This consists of increases in the enzyme activity per centimeter in the in-line ileum, and increases in the enzyme activity per mg protein in the in-line jejunum as well (89).

5.4 Parenteral Nutrition

In normal adult rats that are fed parenterally, not only is there a reduction in mucosal mass and cell proliferation (92, 143, 159, 330), but there is also a reduction in sucrase and maltase activity (92, 216). This reduction in mucosal enzyme activity occurs rapidly as there is a fall in α -glucosidase, alkaline phosphatase and catalase activity within three days (159). When growing rats are placed on parenteral nutrition, sucrase activity is reduced per unit length of the intestine, but the activity per mg protein is not reduced (257). Furthermore, when growing rats are placed on parenteral nutrition after gut resection, then again the sucrase activity in the residual intestine falls, whereas the sucrase activity in those fed by mouth increases (258). In premature human neonates placed on parenteral

nutrition, a reduction in absorptive capacity is observed (162), but again no reduction in enzyme activity per mg of protein can be recorded (319).

5.5 Starvation

In rats and mice, the effect of semi-starvation is to improve the absorptive capacity of the intestine, with improved absorption of glucose and amino-acids (264). When total food deprivation occurs, however, the villi and the crypts reduce in size (10), and reduced absorptive function and barrier function, and a poor generalised immunological state is observed (138, 214, 396). The structural, kinetic and functional changes associated with starvation in the adult animal are all reversible (4, 10).

In the suckling rat, starvation delays the appearance of sucrase and maltase, but also delays the fall in lactase activity, as if the starvation was delaying the physiological adaptations associated with weaning (127, 139, 277).

In children, steatorrhea, diaorrhea, and depressed absorption of many nutrients is found in chronic protein calorie malnutrition (138), and again a poor generalized immunological response is found (340).

6. OTHER FACTORS INFLUENCING INTESTINAL GROWTH AND ADAPTATION

6.1 Pancreatico-biliary Secretions

Pancreatico-biliary secretions are trophic to the villi of the small bowel where either the normal length of the intestine remains (9, 95), or after partial enterectomy (324, 374, 384), but pancreatic secretions are more potent than bile (9). Nevertheless, adaptation will occur in their absence (324). Furthermore, after bile and pancreatic secretions are diverted to the ileum, despite the observations that the ileum undergoes hyper-

plasia when it receives pancreatico-biliary secretions, the jejunal mucosa, now denied those secretions, does not atrophy. To the contrary, the jejunal mucosa actually undergoes hypertrophy with hyperplasia (251). At the same time, there is little change in crypt depth to suggest any acceleration of crypt cell proliferation, so that the increased villus height appears to be the sole cause of the mucosal hypertrophy (251). Therefore, these observations would suggest that the ileal hyperplasia was the result of improved luminal digestion of now unhydrolysed nutrients passing through the jejunum, and that pancreatico-biliary secretions are not in themselves trophic (251), despite claims to the contrary (117, 374, 385). What is more, the apparent trophic effects of cholecystokinin may be nothing more than the stimulation of bile and pancreatic flow. Nevertheless, human pancreatic juice contains EGF, a known stimulator of intestinal mucosal hyperplasia (7, 102, 155, 218, 315, 316).

6.2 Hormones and Growth Factors

(See also Section 5.1.1 for the effects of hormones on absorptive function and its development.)

Studies on the effects of intestinal bypass, parabiosis with cross circulation, and cutaneous parabiosis between two rats (137, 229, 384, 388, 389), all imply the existence of systemic factors that are in part responsible for the intestinal adaptation after intestinal resection (167). Even the simple observations that intestinal hyperplasia occurs proximal to an intestinal resection (54, 234, 275, 381, 382, 389, 397), and that jejunal hyperplasia occurs when dextrose is infused into the ileum (330), support the existence of some form of systemic effect induced by the presence of undigested nutrients in the distal intestine.

When intestine is bypassed, the mucosa undergoes atrophy, but the degree of atrophy depends on the site of origin of the bypassed loop. If jejunum is bypassed, the atrophy is marked and rapid, whereas if the ileum is bypassed there is hardly any response at all (137, 297, 383). These observations support the contention

that luminal nutrition is paramount in maintaining mucosal mass. Nevertheless, when luminal nutrition is absent from the in-line intestine (after starvation or parenteral nutrition), further atrophy will occur in the bypassed loops (67, 92), and this atrophy is inversely proportional to the amount of in-line intestine removed, so that the more in-line intestine that is removed, the less atrophy there is in the bypassed loop (137). Furthermore, when nutrients are infused into bypassed loops, the atrophy is not reversed (6, 189, 190). Therefore, luminal nutrition cannot be the sole participant in the compensatory regeneration seen after intestinal resection, or in the intestine that remains in the luminal stream after bypass. Although, in parabiosis experiments, intestinal resection in one animal of a conjoined pair produces an intestinal response in its parabiotic partner (229, 388), the response after cutaneous parabiosis has been thrown into some doubt (193), and the response after vascular parabiosis involves early increments in DNA synthesis only (388).

After intestinal bypass, the stimulus for the maintenance of mucosal mass in the bypassed loop could be either blood-borne or neural. Bypass studies cannot differentiate between them. The parabiotic experiments, however, demonstrate that there is at least some blood-borne factor. Furthermore, when foetal intestine is implanted under the renal capsule of adult mice, the foetal intestine responds to the same stimuli as the host animal (76), or is influenced by the hormonal status of that animal (188). Furthermore, the intestine of foetal rats, either *in utero* or when cultured, will still respond to the exogenous influence of steroids (184), or the other hormones that will normally influence that intestine *in vivo* (180, 199-202, 255). Therefore, a quest has been made to find a factor, or factors, that might fulfil the role of the blood-borne growth factor, or so-called enterotropic factor (383).

Several gastrointestinal polypeptides have an apparent stimulatory effect on the gastrointestinal tract, and the serum levels

of many gastrointestinal polypeptides rise after intestinal resection (34). Those with demonstrable roles include gastrin, cholecystokinin, and enteroglucagon (34, 35). One further polypeptide, neurotensin, has been found at elevated serum levels in coeliac disease, but its role as a trophic hormone has yet to be elucidated (28, 34). Enteroglucagon has been shown to be elevated in patients with gut resection, and in patients after jejuno-ileal bypass, where large areas of the intestine have been removed from the nutrient stream (34, 308). One further strong pointer for a role for enteroglucagon as a mediator of intestinal hyperplasia in humans was the observation that a patient with a glucagon secreting tumour was found to have intestinal dilatation, and increased villus size (32, 120). Furthermore, when an extract of this tumour was injected into mice, similar intestinal changes were seen (33).

In animal studies, enteroglucagon was found to be present in higher concentrations in the ileal mucosa; was found to be present in high serum and high mucosal levels in the ileum after proximal intestinal resection; was found in relatively low serum and low mucosal concentrations in the jejunum after distal intestinal resection; and was found in very high mucosal and serum levels when rats were hypothermic and hyperphagic, and at very high mucosal levels in the ileum when rats were lactating (167). If enteroglucagon does have a role to play in intestinal adaptation, this distal concentration of the polypeptide may go some way towards helping to explain the poor proximal response to distal small bowel resection. A similar distal concentration has been observed in the mature primate (49), and the adult, neonatal and foetal human (34, 50, 231, 232). In studies correlating crypt cell proliferation with serum enteroglucagon and serum gastrin levels, a good correlation was found between crypt cell production, the presence of nutrients in the distal small intestine, and the serum levels of enteroglucagon (6). No such correlation was found with serum gastrin levels. Furthermore, after intestinal resection, there is an increased concentration of tissue enteroglucagon per unit wet weight of the intestine

(167), and a possible increase in the turnover of enteroglucagon in the mucosa at the same time (282).

Gastrin has also been implicated as one mediator of intestinal adaptation after intestinal resection (167), but only in the fasted rat, as it appears to have little effect on the intestinal mucosa of normally fed rats after an intestinal resection, despite the fact that gastrin will maintain mucosal mass in parenterally fed animals (256). Furthermore, if rats undergo an antrectomy before the intestinal resection, the decreased gastrin levels have no effect on the adaptational response (173, 274). Furthermore, after gastric fundectomy, the increased endogenous gastrin levels do not enhance the post resectional intestinal hyperplasia (274), and there is little, if any, recordable effect when penta-gastrin is administered systemically to normally fed rats after intestinal resection (256). Moreover, after antrectomy in normal rats, the jejunal mucosa may even be stimulated to grow (230). Therefore, after intestinal resection in normally fed rats, gastrin would appear to have little, if any, beneficial role to play (173, 256, 274, 375).

Apart from its known stimulatory effects on pancreatic mass (34), infusions of cholecystokinin are associated with increases in DNA, RNA, and sucrase activity in the jejunum. In addition, in conjunction with antrectomy, cholecystokinin infusion produces an increase in jejunal villus height in normally fed rats. Nevertheless, this could merely reflect the stimulatory side-effects of cholecystokinin on pancreatobiliary secretions (160) which, in turn, stimulate mucosal hyperplasia (9, 68, 324, 384). Furthermore, insulin has been found to stimulate DNA synthesis throughout the intestinal tract (317).

Intestinal hyperplasia is not necessarily the only mechanism whereby intestinal absorption may be enhanced by hormonal factors after intestinal resection. For example, when animals are lactating, there is a placental lactogen dependent increase in the numbers of receptor sites for cobalamin-intrinsic factor com-

plexes, and this increase apparently accounts for the increased absorption of cobalamin in pregnancy (114).

Not only do hormones enhance intestinal hyperplasia, but they may also directly influence the absorption of nutrients within the intestine. This can be demonstrated by auto-transplanting a de-nervated loop of bowel into a dog, and then demonstrating that absorption is improved in that loop during feeding in comparison with absorption during starvation (312). This, however, could also be a receptor effect.

Not only are there systemic stimuli for mucosal hyperplasia after intestinal resection, but there are also non-nutritional, non-pancreatico-biliary luminal growth factors that are present specifically in the milk of suckling animals that may well have marked stimulatory effects on mucosal and longitudinal intestinal growth. Breast milk mitogen and epidermal growth factor have both been found in breast milk in humans (62, 142, 342) and animals (142, 194, 195, 378), and it is possible to speculate that they are there to promote intestinal growth, especially as epidermal growth factor induces intestinal mucosal hyperplasia (61, 63, 79, 80, 102, 315, 316 - see also Chapter 2).

While the gastrointestinal hormones might be expected to exert the greatest effects on the gastrointestinal tract, this is merely surmise, and other hormones, especially those from the adrenals, may play a significant role. For example, mineralocorticoids may be responsible for proximal intestinal adaptation when the loss of distal intestine is associated with fluid losses (347, 379, 380). Furthermore, prednisolone, in pharmacological doses, has been shown to enhance intestinal enzyme activity after gut resection in rats (321), and even androgens and anti-androgens will affect intestinal cellular proliferation (356).

Even at the local tissue level, there appears to be a feedback mechanism whereby the size of the villus determines the rate of crypt cell proliferation. If the villi are tall, then proli-

feration is slow, and *vice versa*. Furthermore, there are inhibitors of crypt cell proliferation which can be extracted from the intestine of one species which will then inhibit crypt proliferation in another species (309, 310).

6.3 Neural Factors

Although the parabiosis and bypass studies have suggested an hormonal factor, one further potential systemic mechanism for the stimulation of mucosal hyperplasia is a neurohumoral feedback (206). In support of this hypothesis are the observations that: vagal deafferentation after enterectomy reduces the mucosal response to the resection (207); noradrenalin increases crypt cell proliferation, and this is reversed by α -blockade; stimulation of the mesenteric nerves produces increased crypt cell proliferation, and cholinergic drugs are inhibitory; adrenaline inhibits crypt cell proliferation, and this is reversed by β -blockade; and, finally, immuno-sympathectomy reduces crypt cell proliferation (91, 354, 355).

Despite all of these observations, when neural stimuli were altered in long-term studies after chemical sympathectomy, there was no decrease in mucosal mass, protein or DNA content in normally fed rats, but chemical sympathectomy could produce long-term reductions in these parameters when rats were fed solely by vein (217).

7. SURGICAL MANIPULATIONS EMPLOYED TO IMPROVE ABSORPTION

7.1 Procedures That Increase Absorptive Surface Area, Including Intestinal Transplant

While the present studies concentrate on the biological aspects and potentials for inducing an increased intestinal absorptive surface area after intestinal resection, there are several mechanical methods that are being attempted to achieve the same

end. There are, essentially, three methods that can be used to increase absorptive surface area. The first is to carry out longitudinal division of a loop of intestine into two narrower strips that can then be sutured up as two tubes (29). This, of course, requires very delicate splitting of the blood supply to the original length of the intestine, so that both new tubes survive. Furthermore, this method does not increase the absorptive surface area immediately, only the length of the intestine and, as some studies have suggested that the numbers of villi do not increase after an intestinal resection (107, 110), absorption might not be improved. Narrowing of the intestine may also so decrease the transit time that there would be less exposure of nutrients to the mucosa. Nevertheless, where such a procedure has been carried out in a human neonate, there seems to have been a good result (37).

The second procedure is to produce a suitable surface for the ingrowth of intestinal mucosa. This has been accomplished by opening the intestine longitudinally, and creating a long elliptical defect in the anti-mesenteric border, then applying the serosal surface of adjacent bowel, or the adjacent abdominal wall, to the defect (30, 224). This allows so-called "neo-mucosa" to migrate in over the serosa, and to form a new absorptive surface. This implies, in fact, that new villi must form to do so. Therefore, the intestinal mucosa must be capable of forming new villi, as has been occasionally reported after experimental intestinal resection (260, 327).

The third potential method is intestinal transplantation but, while this is technically possible in the experimental animal (332-334), intestinal transplantation has failed in humans (332), so that the majority of humans with irreversible malabsorption have been managed with long-term home parenteral nutrition.

7.2 Procedures That Increase Intestinal Transit Time

Surgical procedures have been used to try to alleviate the problems of the short bowel syndrome by the interposition of a reversed loop or a loop of colon within the small bowel. These methods rely on the production of an incomplete intestinal obstruction which, in turn, produces an increased transit time, and prologation of the time of exposure of nutrients to the intestinal mucosa. Again, fine judgement is necessary, to make sure that a complete obstruction, or a stagnant loop, is not produced. The results have often been disappointing (241, 291), although some successes have been claimed (18, 115).

CHAPTER TWO

THE ANIMAL MODELS

The animal used for the majority of these studies was the suckling rat. Apart from the obvious availability, the ease of handling, and the relatively low cost, there are other reasons why the unweaned rat is a particularly suitable animal for this experimental programme.

Before weaning has commenced, the rat is blind, and depends entirely on its mother for nutritional and environmental support. As the suckling rat pup is an atricial animal (147), it makes no attempt to forage from its surroundings until weaning begins at 17 days of age (39, 113, 148, 302). Prior to this, the physiological adaptations necessary for an independent existence have not even begun (147).

This lack of any attempt to forage from the environment makes the suckling rat an ideal animal to study the adaptational response found in the residual intestine after partial small bowel

resection in the neonatal period. As long as the studies are completed before weaning and foraging begins, the diet remains within well defined limits (8). Not only have the major constituents of rat milk been identified (93, 123, 205), but it also appears that the volume of milk is controlled by the mother, no matter what nutritional stresses are placed upon the rat pup itself (147).

Before 17 days of age, suckling rats are incapable of responding to the simple nutritional stress of an enforced fast, as they seem to lack the control mechanisms necessary to increase their milk intake (119). As a result, it is the mother, and not the suckling rat, that determines the volume of milk ingested (147). Even when rat pups are stressed excessively by the detrimental effects of chronic malnutrition, milk volume does not increase to compensate, either when normal dams have to feed abnormally large litters (277), or when chronically malnourished dams have to feed rats in a normal sized litter (127). Therefore, compensatory hyperphagia is unlikely to occur after intestinal resection in the suckling rat pup and, as a result, any observed intestinal regeneration should be the result of a true adaptational response, and not the result of the increased luminal nutrition that would follow any hyperphagia (88, 99, 250, 381).

One of the primary aims of this experimental programme was to determine if the intestine of the suckling mammal could respond to intestinal resection as well as, or better than, the more mature intestine of the weaned animal. (See indications for this study.) As weaning involves an abrupt and marked change in intestinal structure and function that enables the rat pup to adapt from a state of total dependence on milk to total independence (16, 147, 255, 351, 359), the response to intestinal resection before weaning may be entirely different from the response that will be observed after the rat has become independent. Indeed, suckling rats, and other suckling mammals, have no functional intestinal reserve zone in the distal small bowel (44, 108, 359) and, as a result, may well be completely incapable of responding to intestinal resection as effectively as the more mature, weaned rat can (271, 381).

Unfortunately, in previous studies, when intestinal resections have been carried out in the neonatal period, or at least before weaning has occurred, the animals studied have subsequently been allowed to live well beyond the weaning period before they were sacrificed (17, 70, 295). Therefore, the subsequent observations on intestinal adaptation furnish little, if any, information as to the nature and extent of the adaptational response to intestinal resection that occurred only while the animals were still suckling. Despite this, and despite the absence of a functional reserve zone in the distal intestine of suckling mammals (359), the human neonate appears to be capable of a greater functional adaptation to intestinal resection than the adult human being (182, 295, 296, 319, 360). Therefore, as far as possible, experiments on rats in this study were completed before 15 days of age, so as to complete all of the necessary observations before weaning and foraging began.

Previous studies on intestinal resection in the neonatal rat have resulted in an unacceptably high rate of cannibalism (295). When operating on suckling rats, we found that, if an intestinal resection was carried out in the first 9 days of life, the rate of cannibalism was unacceptable. If an older, 14 day old, rat is used, however, the response to intestinal resection may not represent the true neonatal response, especially when the animal has been artificially fed (295). Despite this, we have still chosen the ten day old suckling rat as the main experimental model, as the rat intestine, at this age, has many similarities to the human intestine at birth, and remains morphologically (351) and functionally immature while the rat continues to suckle (16, 147, 255, 359).

As examples, absorptive function in the human is incomplete at birth, as gastric and pancreatic proteolytic enzyme activities are low (13, 192), and fat is incompletely absorbed (108, 192, 368). In the suckling rat, proteolytic activity is also low (13, 16, 255), and in the absence of adult values of pancreatic lipase activity fat digestion relies on lingual lipase (133). As a re-

sult, fat digestion continues well down into the distal small bowel (359).

The patterns of development of intestinal lactase activity are similar in rat and man and, in the suckling rat, lactase activity remains unchanged until weaning begins (147). The pattern of development of α -glucosidase activity, however, is quite different in the two species. Sucrase and maltase activities are present from early on in human foetal development, but these enzymes are absent in the newborn rat, and only appear as weaning begins (16, 255).

From the light microscopic, histochemical and ultrastructural viewpoint, the absorptive and secretory apparatus of the human foetal small bowel is well developed by the middle trimester and, morphologically, the enterocyte of the human foetus at 10-20 weeks gestation resembles that of the suckling rat (213, 192, 351), whereas the rat intestine continues to mature until weaning is complete (255). Therefore, although the intestines of the two species are by no means identical in their developmental patterns, the continued extra-uterine differentiation in the suckling rat appears to make the intestine of that animal comparable with a prematurely born human neonate.

Furthermore, we did not need to artificially feed the suckling rats in our studies, as we found that, with careful handling, even 10 day old suckling rat pups will tolerate major gut resections, and will be accepted back to be successfully suckled by their natural dam (Fig. 2.1), as long as they are allowed to fully recover from the anaesthetic, and as long as all the animals in a litter have undergone surgery. If experimental animals are mixed in a litter with normal animals, then those animals that have undergone surgery will be sought out by the dam and destroyed.

In the initial study in this programme, the experimental animals used were 14 day old, 21 day old and 28 day old rats. As the purpose of this first experiment was to determine if the response



Fig. 2.1. 10 day old rat pups suckling within four hours of intestinal surgery. Note the closed eyes, and the hairless abdomen at this age.

to intestinal resection was quantitatively different before and after weaning, we chose the 14 day old rat pup, just before weaning, the 21 day old rat pup, going through the physiological changes of the weaning process ("the weanling rat"), and the 28 day old rat pup that had just completed the physiological changes of the weaning process. By including the weanling rat, the effect of the nutritional perturbations associated with weaning would be superimposed on those of the intestinal resection itself, and in this way the effects of the two synchronous physiological stresses could be studied. After all, when the human neonate is subjected to a massive intestinal resection, the adaptational response that follows has to be superimposed on the adaptation to extra-uterine life, and the nutritional stress of intestinal resection. During this experiment, however, we found that

the surgery itself accelerated the normal process of intestinal mucosal maturation that occurs at weaning, and the response was most marked in the distal small bowel.

In an attempt to separate the effects of the surgical stress on the one hand, and the effects of increased distal luminal nutrition on the other (removal of proximal bowel allows chyme with a higher concentration of nutrients to reach the distal bowel (85)), we carried out ileal bypass in the 10-12 day old suckling rat pup. When we examined the changes in cellular kinetics and the changes in the mucosal enzyme activities that followed, we found profound differences between in-line and bypassed intestine after only 10 days. Since we had demonstrated such marked changes after only short-term bypass, we followed up this experiment by studying the effects of four weeks of intestinal bypass on longitudinal and mucosal growth of intestine from rats that had undergone bypass at 10 days of age. Both of these bypass experiments demonstrated that the absence of luminal nutrition played an important role in suppressing gut growth and development. Therefore, we went on to attempt to mimic a clinical situation, where luminal nutrition would be absent from the intestinal tract.

When neonates undergo massive intestinal resection, parenteral nutrition plays a major role in their post-operative management. In that situation, luminal nutrition is absent, and a potential paradox exists, where the therapeutic tool that is employed to save the neonate's life may jeopardize the adaptational response by suppressing intestinal elongation and mucosal growth, at a time when the intestine should be growing rapidly (131, 142, 157, 320, 378). When it came to administering parenteral nutrition to suckling rats, however, the task proved technically too difficult. Therefore, as a compromise, the youngest and the smallest rat was used that would survive, first, a 70% mid-small bowel resection, second, ten days of nutrition exclusively by vein and, third, survive all of that in a good nutritional state. The 95g rat at approximately 35-40 days of age was the youngest animal that would do all of that.

That study also demonstrated that luminal factors were also important in the immature animal. Once luminal factors had been shown to be as important in the neonatal and immature animal's response to intestinal resection and bypass as they are in the adult animal's response (85, 382), a search was made for known, enterally presented growth factors that might accelerate or enhance the adaptational response to intestinal resection. Again, the pre-weaned rat is ideally suited to study the *in vivo* effects of intact growth factors.

In the suckling rat, the low acid output (341), and the absence of pepsinogen from the stomach (112), allow for the transit of intact polypeptide macromolecules out of the stomach and into the small intestine without digestion (147). Furthermore, once in the small intestine, immediate digestion by pancreatic proteolytic enzymes does not occur, as pancreatic enzyme secretion is also low during the first 17 days of life (299). As a result, the stimulatory effects that intact growth factors may have on the intestine can be studied in the intact animal without recourse to the formation of isolated intestinal sacs.

Nature has provided at least two situations where growth factors are present in high concentrations in the upper gastrointestinal tract. Firstly, epidermal growth factor (E.G.F.) is present in high concentrations in the saliva that comes from the submandibular salivary gland of the post pupertal male mouse (261, 262). Furthermore, E.G.F. is apparently responsible for the circadian changes in DNA synthesis that occur in the mouse intestine (315, 316) and, when submandibular sialadenectomy is carried out in male mice, the DNA content of the intestinal mucosa falls (218), the somatic growth of the animal slows, and the basic metabolic balance of the mouse is altered (219). Therefore, we carried out submandibular sialadenectomy in male mice, and then subjected them to a 60% mid-small bowel resection, to determine if the adaptational response would be adversely affected.

Secondly, breast milk has been found to promote rapid growth in the intestinal tract (131, 142, 157, 378), and E.G.F. and breast milk mitogen have both been found in high concentrations in breast milk, but only when that milk has come from mothers that have recently given birth. Therefore, colostrum is especially rich in both of these factors and, as lactation progresses, the activity of both of these growth factors and growth promoting activity diminishes (62, 131, 142, 157, 194, 195, 320, 342, 378). Therefore, after subjecting 10 day old suckling rats to a 60% mid-small bowel resection, we fostered them out to dams that had already been lactating for 3 days, or 10 days, or 20 days, and studied the adaptational response within each group. To act as a control, we gavage fed a similar group of rats that had also undergone intestinal resection, to determine if breast milk in general was superior to commercial milk formulas for inducing a regenerative response to intestinal resection.

One further growth factor that has received attention recently is the polypeptide hormone gastrin. Gastrin appears to be capable of stimulating mucosal growth throughout the gastrointestinal tract, whether it acts through the blood stream (175), or whether it acts through the intestinal lumen (176). Nevertheless, there are doubts as to its efficacy in non-fasting rats (see Section 6.2 of the Literature Review). Therefore, we carried out partial gastrectomies in the 10 day old suckling rat in such a way that one group lost the antrum, another group lost the fundus, and a third group lost the rumen. This produced one group of rats with a low serum gastrin level, one group of rats with a high serum gastrin level, and a third group of rats with a normal serum gastrin level, but a similarly reduced gastric volume. After this had been achieved, we followed their intestinal development through the weaning period.

Not only does the lack of protein digestion in the upper GI tract allow intact growth factors through to the small bowel, but it also allows intact antigens through (147). However, one of the major physiological changes that takes place at the time of wean-

ing is the maturation of mucosal barrier function, so that antigenic material is almost completely excluded (357, 363). Therefore, to determine if the intestinal resection altered the intestinal permeability to macromolecules by damaging the mucosa, or whether the systemic response to surgical stress induced precocious mucosal barrier function, we gavaged fed bovine serum albumin to suckling rats 5 days after partial small bowel resection.

THE EXPERIMENTAL STUDIES

SECTION ONETHE EFFECT OF AGE AND WEANING

CHAPTER THREE

THE EFFECT OF AGE AND WEANING AT THE TIME OF
INTESTINAL RESECTION

The response to intestinal resection in suckling animals should be of great interest to paediatric surgeons. Yet little is known about the neonate's early adaptational response to intestinal resection. What is more, the functional and kinetic immaturity of the intestine in the neonatal animals would suggest that the intestine is quite unsuited to mount an effective adaptational response, as the neonatal intestine normally has a slow cell turnover (202, 339) and slow crypt cell proliferation (11), both of which have to increase rapidly in the immediate period after intestinal resection (381). During the neonatal period, however, the intestine is functionally well adapted to the absorption and digestion of breast milk. The extent and nature of this particular adaptation is, however, so extensive that an effective response to intestinal resection seems unlikely.

First of all, while an immature animal is still suckling, there is no functional reserve zone in the distal small bowel (41, 108, 359). Both the jejunum and the ileum are required for absorption of nutrients, and even then absorption is incomplete (108, 359). The situation is entirely different in the adult animals, however, as there is a distal reserve zone which is normally unstimulated by the undigested nutrients and by the pancreatico-biliary secretions that are normally present in the jejunum (9, 384). It is this uncommitted distal reserve



zone that responds so well to the increased concentrations of luminal nutrients and pancreatico-biliary secretions that reach the distal intestine when proximal small bowel is removed; and, in adult animals, it is this reserve zone that appears to be responsible for the improvement in function after intestinal resection (85, 271, 381).

The next major difference between adult and neonatal intestine is that more than 50% of cell production in the neonatal intestine is destined for structural growth (11). Therefore, recruitment for the extra demands of an adaptational response may be impossible. Furthermore, cell production in the neonatal intestine is much slower than in the adult animal (11, 151, 239), and the marked increases in cell production seen in the adult animal after intestinal resection (376, 381) may not occur in neonatal animals. On the other hand, this slow cell proliferation may be to the neonate's advantage, and may act as a functional reserve. Therefore, contrary to expectations, a greater potential could exist for an adaptational response to occur in the neonate, and such potential reserve for cell production could explain the apparently improved response to intestinal resection in the human neonate (252, 295, 296, 318, 319).

Because of these differences between the adult and the neonatal intestine, and the possible potential they could have for modifying the adaptational response, we set out to determine if the intestinal response to intestinal resection before weaning started was similar to the intestinal response to intestinal resection after weaning was complete. In addition, we studied the adaptational response while the physiological upheavals associated with the weaning process were under way. For this purpose, we subjected 14 day old, 21 day old and 28 day old rats to 60% mid-small-bowel resection.

The fourteen day old suckling rat is still totally dependent on its mother for support, and will continue to suckle for at least another nine days (148). Despite this dependency, foraging

begins to occur at approximately seventeen days of age, and breast milk intake begins to fall significantly by 20 days of age (147). Therefore, there is a potential six day period for observation during the suckling period before the diet changes significantly, if intestinal resection is carried out at 14 days of age, and sacrifice is carried out at 20 days of age. As the structural response within the residual intestine of the adult rat takes between 6 and 12 days to reach its maximum (381), these six days may well be sufficient to accommodate a maximum response to intestinal resection.

The twenty-one day old rat has already started to forage, and the intestinal mucosa would have started the redifferentiation process associated with weaning (255). If it, too, were sacrificed at six days after an intestinal resection, this would allow observations on intestinal adaptation to be made at a time when the intestine was already committed to another physiological change. The two processes might be mutually exclusive, so that the adaptational response would be in abeyance while intestinal maturation was occurring. Or, the added stimulus of another physiological stress might accelerate mucosal maturation, as has already been observed during other physiological stresses in the suckling rat (43, 210, 211).

At 28 days of age, the physiological changes associated with weaning are nearing completion. If surgery were carried out at this age, and if sacrifice were carried out six days later, these physiological changes would be complete, for the 34 day old rat is now totally independent (147). This age of animal, therefore, would be best suited to compare the response of the immature pre-weaned rat with the immature, but post-weaned, rat. In this way, all of the animals in this study would be growing rapidly at the time of resection, and at the time of sacrifice, and the only difference between them would be the intervening intestinal redifferentiation that occurs at weaning. None of the changes associated with ageing would be involved (69, 76, 97, 110, 185).

Once the early response to intestinal resection was determined, a study was also made of the longer-term response to intestinal resection, again using these three age groups at the time of resection. As the structural response to intestinal resection in the adult rat has reached its maximum 12 days after an intestinal resection (381), we sacrificed a further three groups of rats 12 days after they had undergone small bowel resection at 14, 21 and 28 days of age, to determine if the maximum response had already occurred 6 days after the resection, or whether further adaptation was still taking place 12 days after the gut resection. Although this would take the youngest rat out of the suckling period, it would have the added advantage of demonstrating the effect that mucosal redifferentiation would have, when it was superimposed on an adaptational response that was already occurring. To determine if a further adaptational response to intestinal resection was possible after 12 days in these immature animals, we sacrificed a third series of animals 24 days after the intestinal resections. In the whole study, therefore, rats were operated upon at 14, 21 and 28 days of age, and each of these three groups of rats was further subdivided so that sacrifice was carried out at 6, 12 and 24 days later.

METHODS

Male Wistar rat pups (n=120) were used. Three days after birth, rat litters were reduced to a maximum of ten pups to a litter. For this experiment, before weaning at twenty days of age, all the animals were housed with their dams, whether or not they had undergone surgery. At twenty days of age, all rats were separated from their dams, and were either caged individually, or sacrificed. Rats were housed in a light-cycled isothermic room, and were fed *ad libitum*, whether they were suckled or weaned. The weaned and weanling rats (those rats that would normally be going through the process of weaning) were fed on standard Purina laboratory rat chow. Prior to surgery, suckling rats (n=48, 14 days old) were left undisturbed with their dams. Weanling rats (n=39, 21 days old) were separated from their dams at twenty days of age,

and had been allowed chow *ad libitum* up until 12 hours before surgery. They were also allowed water only for the 12 hours prior to surgery. Intestinal resection or transection was carried out at 14, 21 and 28 days of age. The rats undergoing intestinal transection only were to act as controls that had undergone the stress of intestinal surgery but still had all of their intestine *in situ*.

After induction of light ether anaesthesia, all of the animals received subcutaneous injections of 0.9% saline solution in 5% dextrose equivalent to 50 ml per kg (or 5% of their body weight). This solution also contained 1000 units of crystalline penicillin per ml, and the rats also received 2.5 mg of kanamycin subcutaneously. At surgery, the entire length of the jejunum and the ileum was measured along its anti-mesenteric border by gently stretching it along a measured silk thread, care being taken not to over-stretch the intestine (any variation introduced by this method would be equally applied to both groups of animals). Surgery was performed with the aid of a dissecting microscope, and consisted of a 60% mid-small bowel resection or a small bowel transection (Fig. 3.1). The proximal forty percent of the jejunum and the distal forty percent of the ileum remained after intestinal resection. Transection and reanastomosis of the intestine was carried out at the same point proximally, and a marking suture was placed in the anti-mesenteric border of the intestine at the same point that the resection had finished distally. Single layer end-to-end anastomoses were carried out with interrupted 7.0 prolene. The abdomen was closed with 6.0 silk.

After surgery, and after fully recovering from the anaesthetic, 14 day old animals were returned to their dams, and were seen to suckle normally. Weanling and weaned animals were allowed water for another 12 hours, and were then allowed access to chow *ad libitum* until the time of sacrifice. To avoid cannibalism of the rat pups that were returned to their dams, all the animals within any one litter underwent surgery, as mixing these animals with normal litter mates resulted in the destruction of those that had been operated upon.

At sacrifice, rats were decapitated. The entire length of the jejunum and the ileum was removed rapidly and measured suspended under 2 g tension applied to the distal end. The most proximal 5 cms of jejunum was removed for biochemical assay (Fig. 3.1). This was washed thoroughly in ice cold saline solution to remove all debris, both before and after eversion over a glass rod. The next one cm of the jejunum was opened longitudinally, immediately backed onto a porous paper card with the mucosal surface uppermost, then placed in 10% buffered formalin solution. Similar specimens of the ileum were removed for biochemical assay and histology from 5-10 cms and from 10-11 cms distal to the anasto-

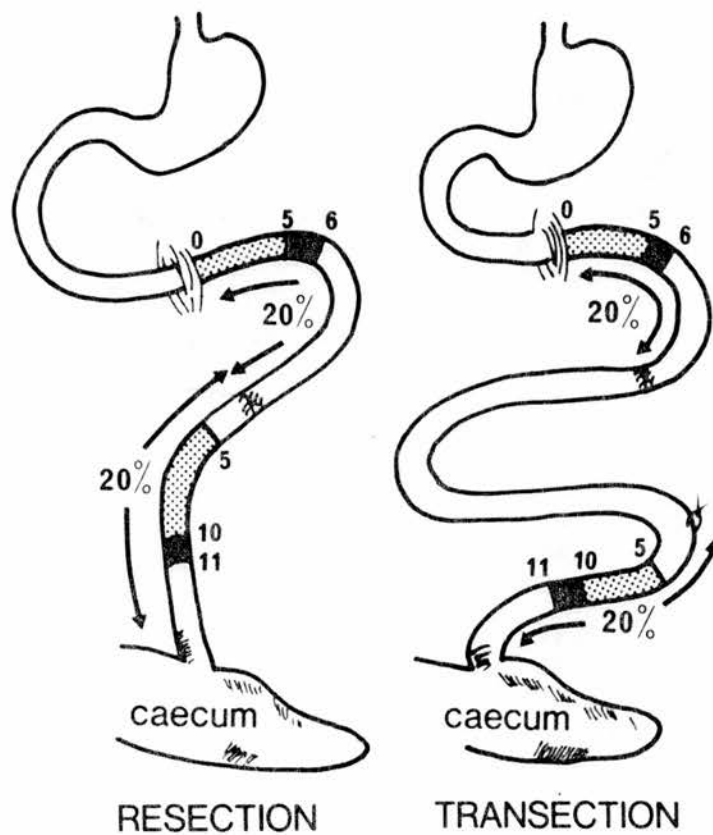


Fig. 3.1 Intestinal resection and transection. Sixty percent of the mid-small bowel has been removed, as in the left-hand figure, or the same length of bowel was demarcated by a proximal intestinal transection with immediate re-anastomosis and distal marking suture, as in the right-hand figure. Shaded areas represent specimens taken for biochemistry. Solid areas were taken for histology. Numbers represent distances in centimeters.

mosis in those rats that had undergone intestinal resection and distal to the marker suture in those that had undergone intestinal transection (Fig. 3.1). Attempts at mucosal scraping resulted in complete disruption of the gut in these young animals. Therefore, the full thickness of the intestine was used for biochemical assay. These specimens were stored at -20°C in 5 ml of 10 mM sodium phosphate solution at PH 6.0. The storage solution also contained 0.002% triton X-100. For the methodology for the biochemical estimations and histological examinations for this experiment, see Appendix One. In this and other experiments, the disaccharidase activities were expressed per mg DNA, so that an estimate of the activity per cell would be obtained, rather than an estimate per unit of protein (87, 116). If enzyme activities are expressed per unit of protein, then fluctuations in the nutritional state of the animal could spuriously alter enzyme activity by altering protein levels in the cells (162). The DNA content, on the other hand, is constant for diploid cells (362). Furthermore, this form of expressing the results was used to avoid confusion arising from the changes in cell size associated with the normal process of weaning (201), and the changes in cell size that are associated with intestinal resection (324, 376).

Students t test was used to estimate the statistical analysis of the results in these experiments, unless otherwise stated.

RESULTS

One hundred of the one hundred and twenty infant rats survived the surgery. Rats lost weight for the first two days after gut resection, whereas those undergoing intestinal transection did not. If the rats were weaned at the time of intestinal resection, they went on to gain weight more rapidly than if they were still suckling or weaning when the intestinal resection was performed (Fig. 3.2). As a result, the difference in weight between those rats undergoing intestinal resection and those undergoing intestinal transection at 28 days of age had disappeared by the twelfth post-operative day. In those rats that had undergone surgery at

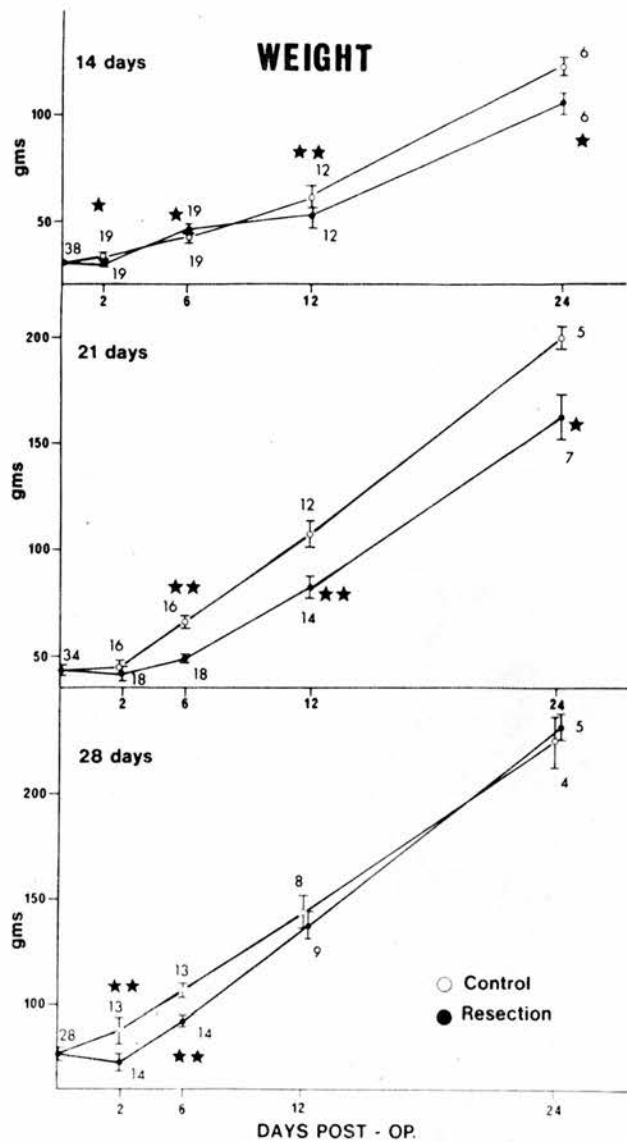


Fig. 3.2 Animal weight. Rats that had weaned at the time of intestinal resection (28 days old) returned to normal weight within 24 days. Those rats that had not weaned at the time of intestinal resection did not regain normal weight for their age during the study period. One asterisk* $p < .01$ resection v transection; two asterisks* $p < .001$ resection v transection.

14 or 21 days of age, however, the difference in body weight after intestinal transection or intestinal resection did not disappear during the study period.

STRUCTURAL CHANGES

By six days after an intestinal resection, there was a consistent response in the intestinal mucosa distal to the resection, but not proximally. The ileal villi were consistently taller than those in animals that had undergone intestinal transection, no matter at what age the intestinal resection was performed (Fig. 3.3). The difference in villus height did not increase further at 12 or 24 days after surgery in any of the age groups studied and, although an increase in villus height was consistently demonstrated downstream of an intestinal resection, there was only an inconstant increase in the villus height in the jejunum of the suckling rat, and an increase in the villus height in the jejunum of the weaned rat (Fig. 3.3).

The response within the crypt was far less constant than the response of the villus. Within the crypts, a significant response to intestinal resection was only seen in the youngest and the oldest rats in this study. Unlike the response in the villus compartment of the mucosa, the response in the crypt compartment did occur both proximal and distal to the resection. The crypt depth in the ileum of the youngest rats (those that were suckling at the time of intestinal resection and sacrifice), was greater than the crypt depth in the ileum of similar animals that had undergone intestinal transection only (Fig. 3.3 - inverted histograms). This difference in crypt depth then abruptly disappeared until the rat reached 45 days of age (the oldest animals at sacrifice). Although there was no significant increase in the crypt depth proximal to an intestinal resection in the youngest rats at sacrifice, there was such an increase in the crypt depth 24 days after an intestinal resection in those animals that were fully weaned at surgery at 28 days of age (Fig. 3.3) (the oldest animals at sacrifice).

The increases in the crypt depth were accompanied by an increase in the number of mitoses per crypt column and, six days after an intestinal resection in the youngest rats, the numbers of cells undergoing mitoses in the crypts was significantly greater than in the same aged rats undergoing intestinal transection alone

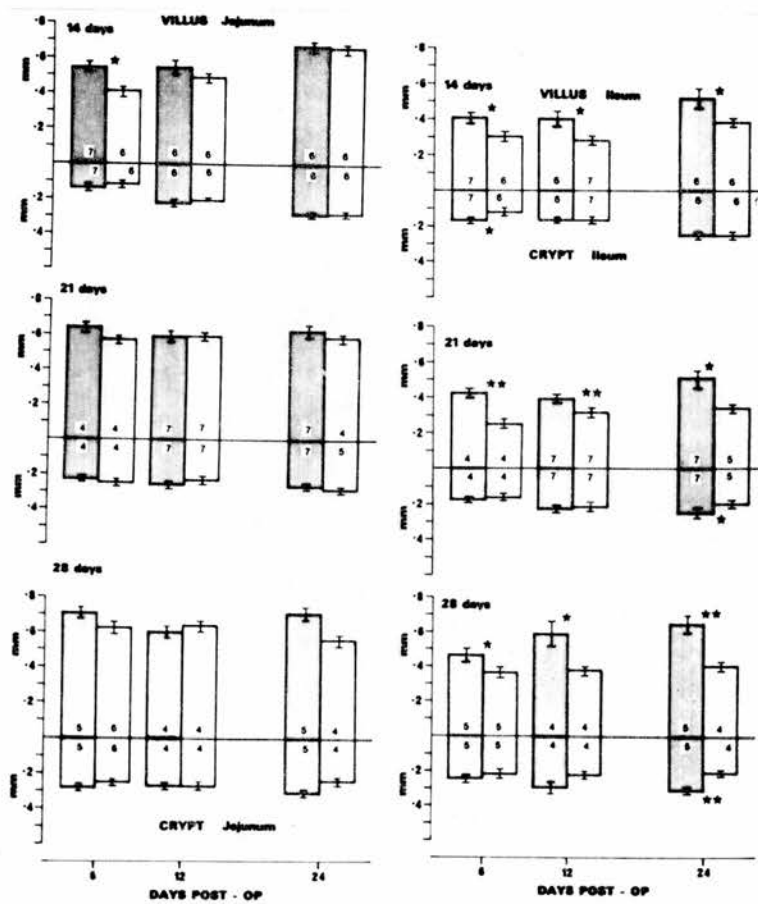


Fig. 3.3 Villus height and crypt depth. Intestinal resection produced a persistent increase in villus height in the intestine distal to the anastomosis (right-hand figure), whereas there was only a response in the proximal intestine in the suckling rat 6 days after intestinal resection, and 24 days after intestinal resection in the weaned rat (left-hand figure). Ileal crypt depth increased in the suckling rat 6 days after intestinal resection, and in the weanling and weaned rat 24 days after intestinal resection. Jejunal crypt depth only increased in weaned rats 24 days after intestinal resection. Significant values as in Fig. 3.2.

(Fig. 3.4). Once more, this difference was only present in the suckling rat six days after resection, and once more reappeared in the oldest animals 24 days after partial enterectomy. These increases in the cell mitoses per crypt column were seen both proximal and distal to the intestinal resection.

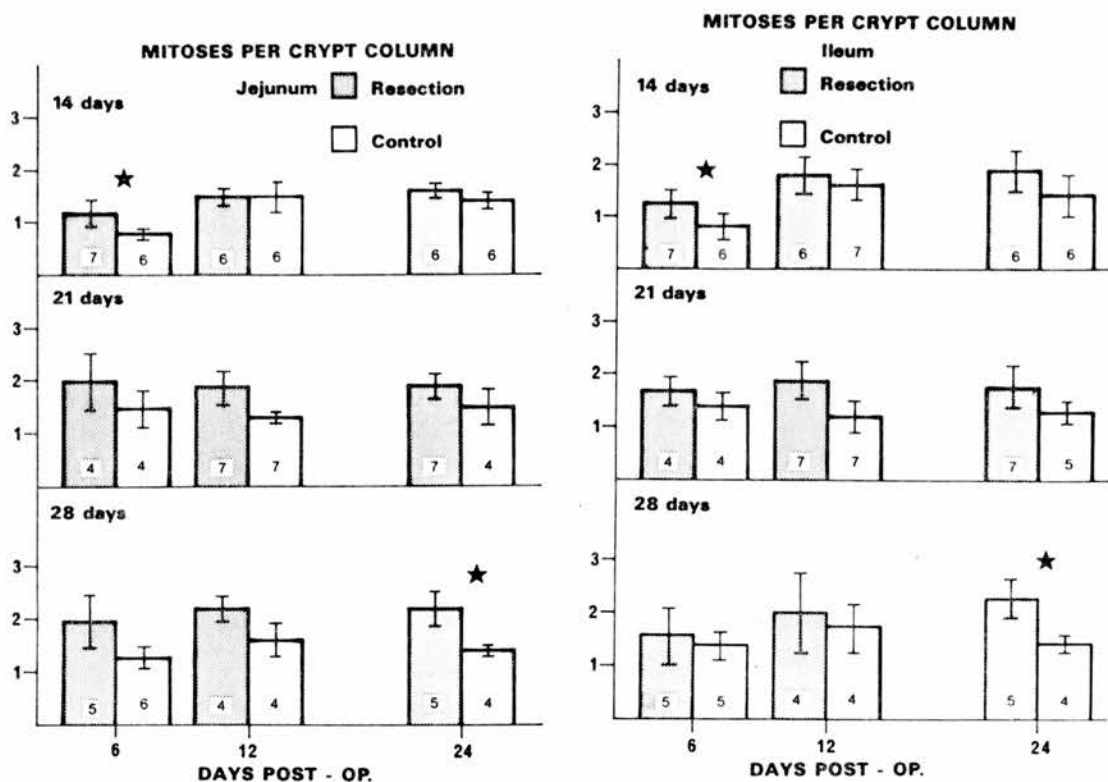


Fig. 3.4 Mitoses per crypt column. Significant increases in mitoses per crypt column were only seen in the suckling rats and the oldest rats in this study, both proximal and distal to the anastomosis. Significant values as in Fig. 3.2.

BIOCHEMICAL CHANGES

Mucosal Hyperplasia

The structural changes were accompanied by biochemical evidence of cellular hyperplasia as the DNA content of the ileum increased after intestinal resection in all age groups, and at all of the sacrifice points recorded (Fig. 3.5). The differences between those animals undergoing intestinal resection and those undergoing intestinal transection did not reach significant values until 12 and 24 days after surgery, however, and only then in those rats that had undergone intestinal resection at 28 days of age. In the proximal intestine, there were no differences in the intestinal DNA content between those animals undergoing intestinal resection and those undergoing transection.

The intestine of all of the rats elongated after intestinal surgery, but the elongation after intestinal resection was virtually identical to that seen in rats undergoing intestinal transection alone (data not shown).

Mucosal Enzyme Activity

Mucosal enzyme activities were altered by the intestinal resection.

Sucrase activity per centimeter increased in all of the animals undergoing intestinal resection, with one exception (Fig. 3.6). The sucrase activity per centimeter failed to increase in the ileum of the suckling rat six days after an intestinal resection. In all the other rats, however, a significant increase in the sucrase activity per centimeter was observed in the distal small bowel at all stages after intestinal resection. In the proximal small bowel, no such differences were observed.

Increases in the sucrase activity per cell were similar to those per centimeter, and were seen in the ileum early after intestinal resection, but only in those rats that would normally be going through the physiological changes associated with the wean-

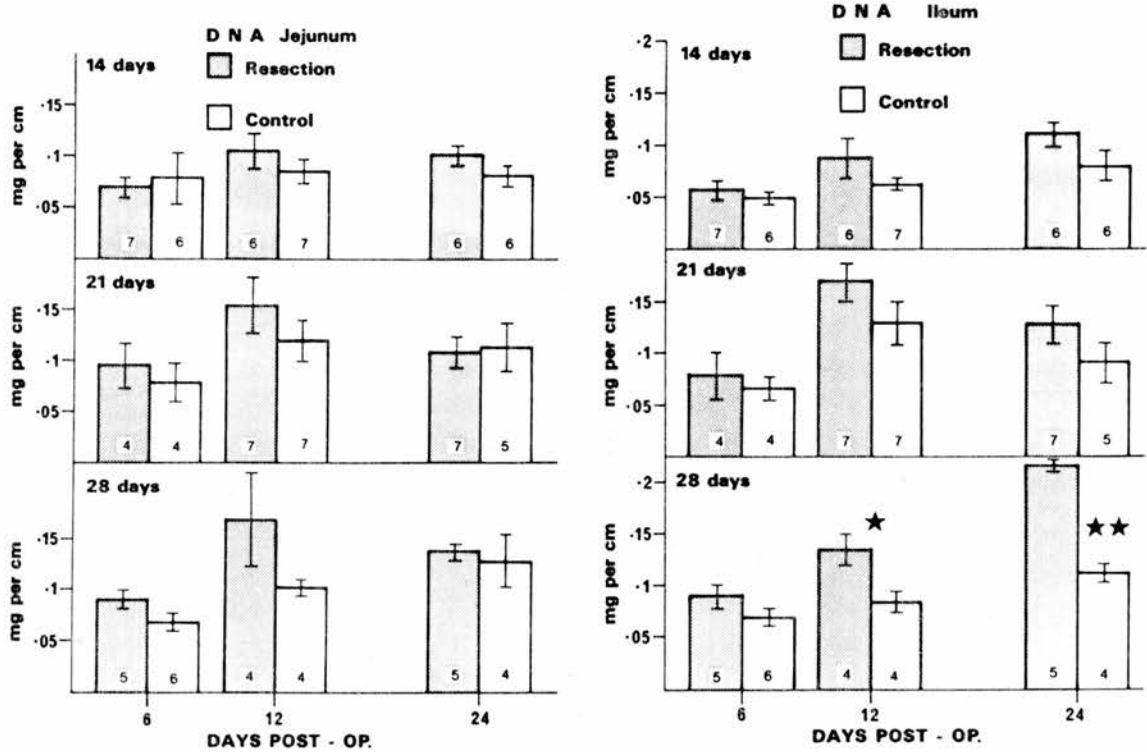


Fig. 3.5 Total DNA content of the intestine. Significant increases in DNA content only occurred in the distal intestine in rats that had been weaned at the time of intestinal resection. Significant values as in Fig. 3.2.

ing process, and those that had weaned (Fig. 3.7). No such increase in sucrase activity occurred proximal to the intestinal anastomosis.

The lactase activity per centimeter abruptly decreased, and virtually disappeared (Fig. 3.8). Significant lactase activity was only found in the 20 day old suckling animal and, after intestinal resection, even that activity was markedly reduced (Fig. 3.8). The lactase activity per mg DNA followed the same pattern

of response (Fig. 3.9). Since the lactase activity in both the jejunum and the ileum fell to almost undetectable adult levels by 26 days of age in all of the animals studied, only the response in the suckling rat is recorded here.

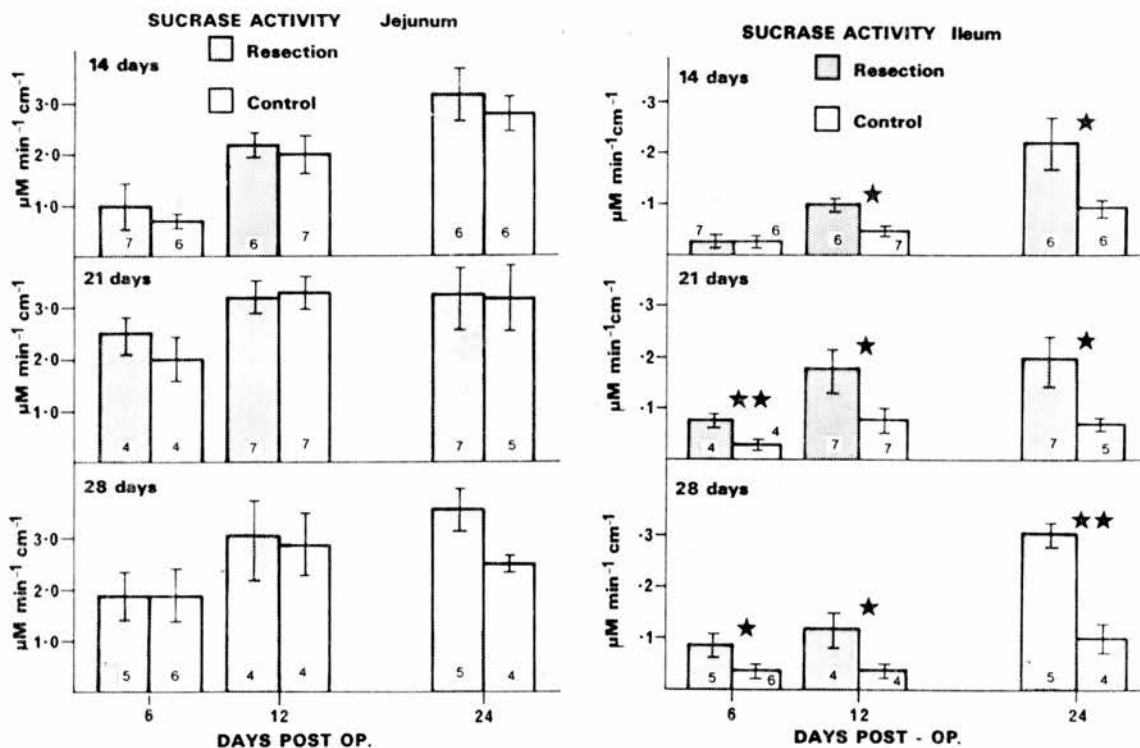


Fig. 3.6 Sucrase activity per centimeter. Sucrase activity per centimeter increased in the distal intestine after intestinal resection. Significant values as in Fig. 3.2.

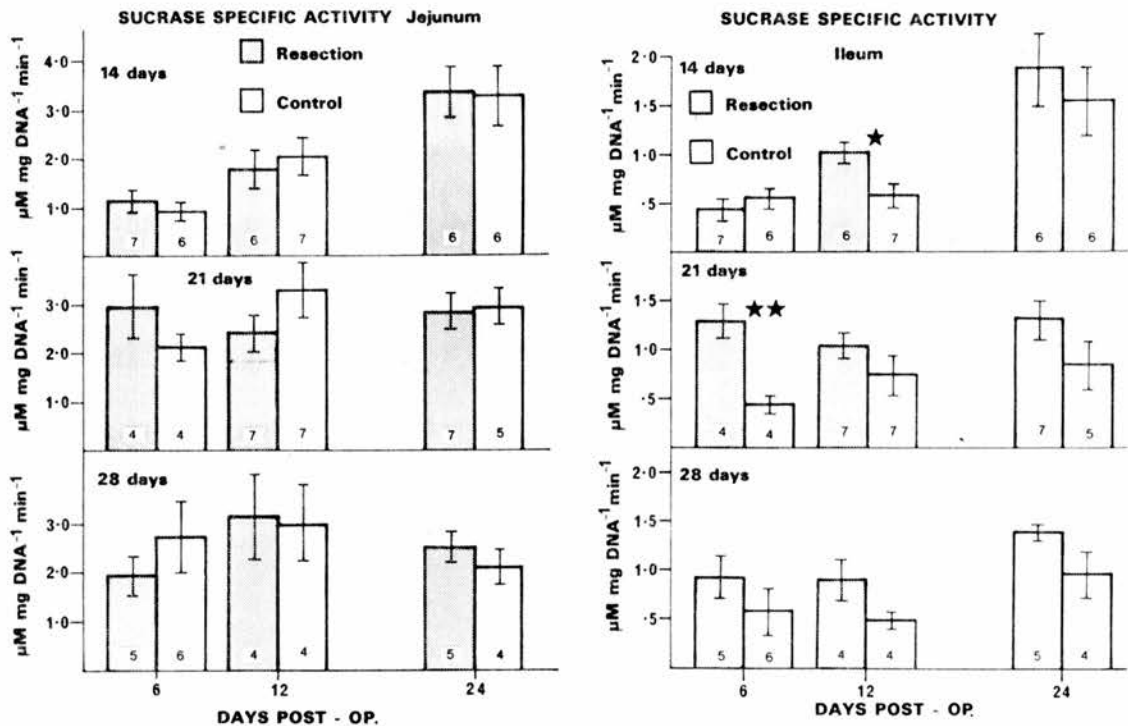


Fig. 3.7 Sucrase activity per cell, expressed as sucrase activity per mg DNA (sucrase specific activity), increased in weanling rats after intestinal resection whether they had undergone surgery at 14 days of age or at 21 days of age. Significant values as in Fig. 3.2.

DISCUSSION

Despite its immaturity, the small intestine of the suckling rat demonstrated the capacity to mount a rapid response to intestinal resection, but the results were partly obscured by the synchronous effects of an adaptational response and the normal changes associated with weaning.

The one consistent structural response to intestinal resection in suckling, weanling and weaned rats was the increased distal villus height, thus demonstrating that the immature intestine of the suckling rat could undergo compensatory mucosal growth, despite the reported absence of a functional and a morphological reserve zone distally (11, 359). As many of the other changes

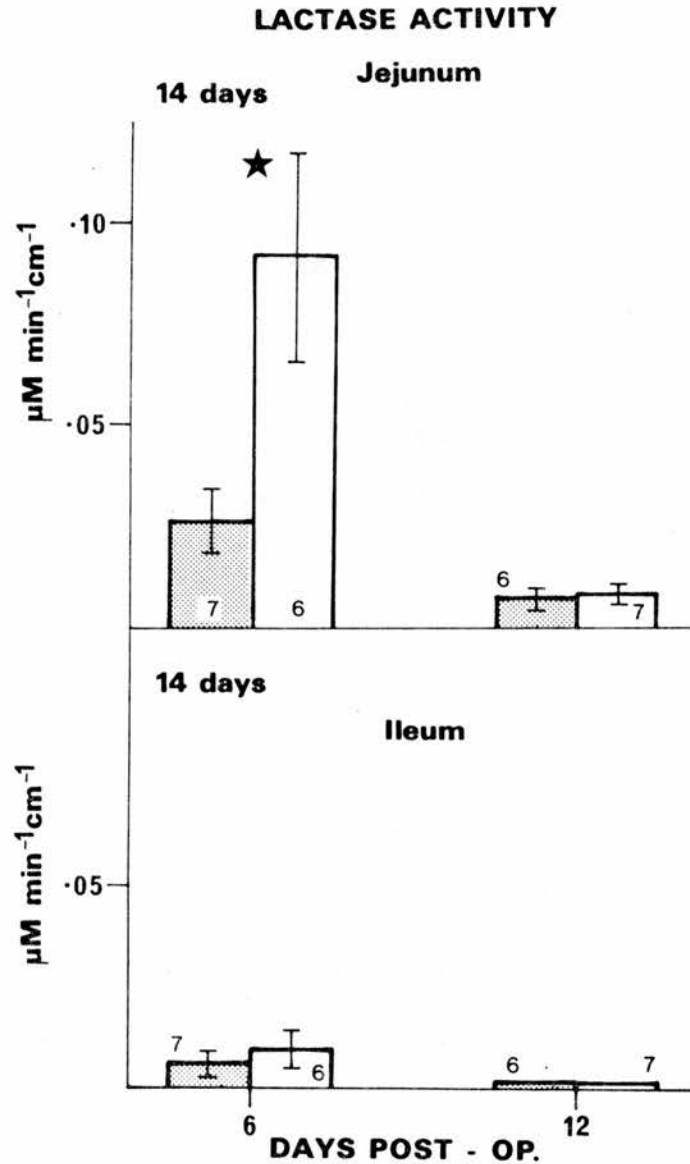


Fig. 3.8 Lactase activity per centimeter. In the 14 day old rat, intestinal resection induced an early decrease in lactase activity in the jejunum. Significant values as in Fig. 3.2.

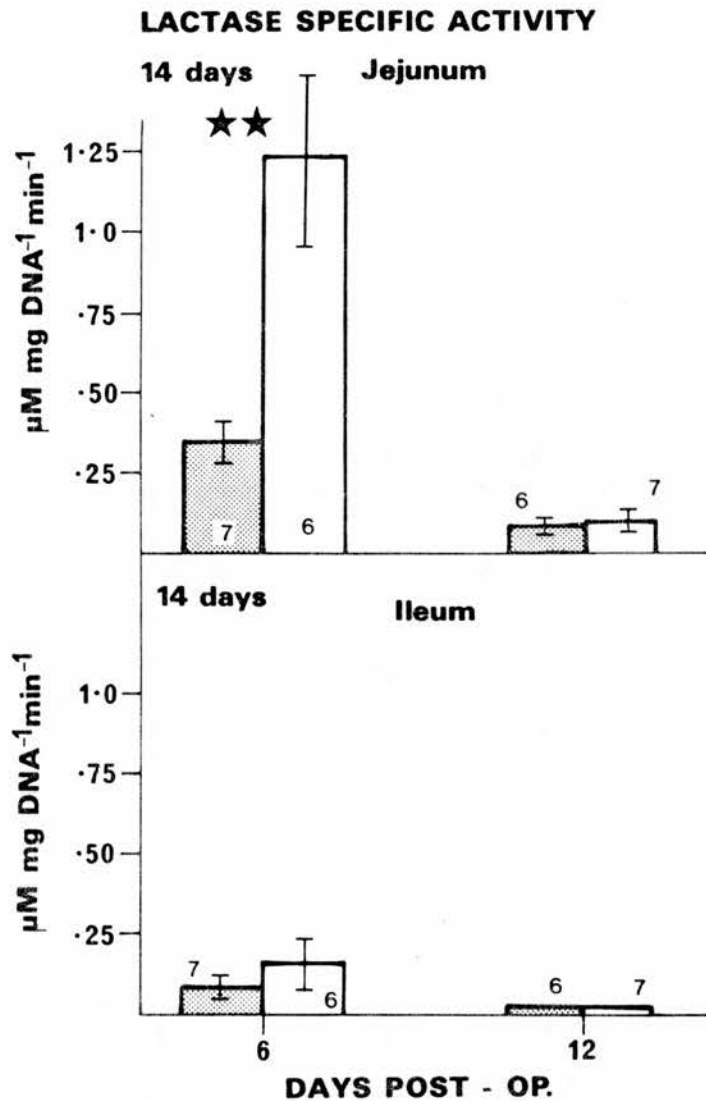


Fig. 3.9 Lactase activity per cell, expressed as lactase activity per mg DNA (lactase specific activity). Intestinal resection induced an early decrease in lactase specific activity in the jejunum of the 14 day old rats. Significant values as in Fig. 3.2.

that occurred in the residual intestine in this experiment appear to represent early mucosal maturation, it is possible that this increase in villus height could also be part of an early mucosal maturation. At weaning, however, the villi normally grow slowly (151), so that the abrupt increase in the villus height recorded

here is probably an adaptational response, but could be an acceleration of the normal changes of weaning. While this particular structural response to intestinal resection in the villus compartment of the intestinal mucosa was quite distinct, the anatomical response in the crypt compartment was obscured by the effects of mucosal maturation.

The response in the crypt appeared to be biphasic. Six days after intestinal resection, there was an increased crypt depth and number of mitoses per crypt column, which was more marked distally. As weaning commenced, however, increases in crypt size and cellular proliferation also occurred in rats that had undergone intestinal transection, effectively obscuring, or even precluding, any response that there might have been to the intestinal resection. This apparent "catch up" in the crypt activity of the rats undergoing intestinal transection was probably the normal physiological changes of weaning occurring slightly later in this group of animals. Once the physiological perturbations associated with the weaning process were complete, however, the effect of the intestinal resection was once more unmasked, and crypt depth and mitotic rates were again significantly greater in those animals that had undergone intestinal resection. As in mature rats, the response within the crypt compartment in these immature growing rats occurred both proximally and distally (381, 383, 384), whereas the response within the villus compartment was more marked distally (85, 86, 381).

While it is tempting to speculate that the earliest response in the crypt compartment of the youngest rats was an adaptational response to the intestinal resection whereby more cells were being produced, it is equally possible that this increased crypt activity was no more than an accelerated mucosal maturation induced by the greater metabolic and surgical stress (210, 211) of the intestinal resection. Therefore, for the 14 day old suckling rat at least, these results have failed to demonstrate a clear-cut response to intestinal resection within the crypts of Lieberkuhn. There was, however, a more clear-cut response once the weaning period was over.

Although after intestinal resection the increase in the ileal villus height, the increase in the crypt depth and the number of mitoses per crypt column would suggest a large increase in the number of cells per centimeter of intestine, the DNA content failed to increase accordingly until the weaning period was over. This suggests that some degree of cellular hypertrophy had occurred to account for the increased villus height in those rats undergoing intestinal resection during the weaning process. Cellular hypertrophy could also be the result of early mucosal maturation, as cellular hypertrophy is one of the physiological changes that occurs at weaning (201, 339).

In distinct contrast to the adult rat (269, 307), additional intestinal elongation over and above normal growth does not appear to be part of the compensatory response in immature rats. Possibly, the bowel is already elongating at its maximum rate in this age group.

Not only did the weaning process obscure the crypt's response to intestinal resection but, unfortunately, the changes in enzyme activity after intestinal resection were also obscured by the normal changes that occur in enzyme activity at weaning. The expected response in the mature rat is an overall increase in enzyme activity per centimeter, brought about by a vast increase in the number of cells per centimeter, and not by an increase in enzyme activity per cell (319, 376, 381). To the contrary, in the adult rat, enzyme activities in the enterocyte may even fall after intestinal resection (376).

Therefore, the increase in the sucrase activity per centimeter was expected after intestinal resection and, as expected, was seen in all of the rats with sucrose in their diets, as sucrase is an inducible enzyme (149). At the same time, however, the sucrase activity per cell rose in the ileum after an intestinal resection in suckling and weanling rats; quite the opposite response to that which would be expected in the adult rat (376). This partly unexpected response may also be explained on the basis of precocious mucosal maturation, however. Normally, sucrase spe-

cific activity rises sharply from approximately 15 days of age, to reach a maximum at 40-60 days of age (147). In this study, after the stress of intestinal resection, a precocious rise in the ileal sucrase activity was apparent in animals that had both sucrose in their diet and were at an age when the physiological changes associated with the weaning process would be expected to occur. What is more, a similar early rise in sucrase activity has been demonstrated after the stress of gastrostomy (211), suggesting that surgical stress alone can induce these enzyme changes early.

In this study, the suckling animals demonstrated one more apparent paradoxical response to intestinal resection. Instead of the lactase activity increasing after partial enterectomy, it abruptly disappeared; hardly an effective functional response at a time when the major source of carbohydrate is lactose. Again, this change can be explained on the basis of precocious mucosal maturation. Lactase normally begins to fall at 15 days of age in the suckling rat, and reaches the normal low adult levels by 25-28 days of age (147, 151). Intestinal resection apparently induces that change early.

The abnormally early changes in enzyme activity, crypt depth and crypt cell proliferation that have been recorded here have all been produced artificially by steroids (150), thyroxine (181, 255), and also surgical (211) and metabolic stresses (43). Therefore, it is possible to speculate that the additional stress of the intestinal resection induced these changes earlier than intestinal transection, and it is also possible to speculate that these changes were mediated by the hormonal response to the intestinal resection. Similar, but less marked, changes may have occurred after the intestinal transection, but the pattern of mucosal maturation in the control animals did not differ markedly from the previously reported normal pattern (147, 151), and those undergoing intestinal resection consistently had a more marked response than those undergoing transection.

This study demonstrated that the response to intestinal resection in the suckling, the weanling, and the weaned but immature rat, was rapid. The response in the villus compartment of the mucosa was quite distinct. The response in the crypt, and the changes in mucosal enzyme activity, were partly obscured. Nevertheless, the total activity of sucrase per centimeter rose after intestinal resection in all of the animals that had sucrose in their diet. As this increase in sucrase activity was accompanied by a synchronous increase in the villus height, these changes would infer that there was increased absorption of sucrose (85). The early disappearance of lactase activity might suggest that, after surgery in the human neonate, the frequently observed lactose intolerances (152) could be the result of early mucosal maturation bringing about a drop in lactase activity that should normally occur between one and four years of age (14).

This study also demonstrated that there are differences between the intestinal response to intestinal resection in the adult rat and those recorded here. Sucrase activity per cell rose in weaning rats, and there was no compensatory elongation of the intestine. There were also similarities. As in the adult rat, the major response to the intestinal resection was seen downstream of the resection, although there was some response proximal to the resection, in the crypt, and also a decrease in jejunal lactase activity.

In previous studies of adaptation to intestinal resection, the major controlling factor in the downstream response was thought to be the increased luminal nutrition that follows the removal of proximal bowel (151). The proximal changes were thought to be the result of a systemic, humoral or neuroendocrine response (206, 347, 354, 383). A similar systemic response might explain the precocious mucosal maturation seen here, in much the same way that corticosteroid injections can produce similar precocious ontogenic changes in intact suckling rats (150, 255).

Whatever the cause of the response recorded here, it was clear from this initial study that the animal model would have

to be altered radically to carry out any further investigations into the effect of intestinal resection in neonates, so that the confusions caused by the simultaneous occurrence of adaptation and maturation would be avoided. Furthermore, the stress of intestinal transection itself may well have produced similar, but less marked, mucosal maturation, so that any further study would require more adequate controls (see Chapters 5, 9 and 10).

In summary, when animals are suckling, the specialized adaptation to breast feeding, and the slow cyto-kinetics of the immature intestine, may preclude an effective adaptational response to intestinal resection. Nevertheless, when suckling rats were subjected to intestinal resection, sucrase activity was greater, and lactase activity less, than in the distal intestine of control rats undergoing intestinal transection. The villi in the distal gut elongated, and crypts became deeper, as the mitotic activity increased.

When weanling rats were subjected to resection, the physiological changes associated with weaning partly obscured the adaptational response. Villi were taller, and sucrase activity increased, but evidence of increased cell proliferation over and above that occurring in the controls was not found. Lactase activity had disappeared in both groups of rats by this age.

When rats that had completed the weaning process were subjected to intestinal resection, the response was similar to that in adult rats, with increases in villus height, crypt depth, crypt cell proliferation, DNA content, and sucrase activity per centimeter.

The response to resection, although different, was marked in suckling and in weaned rats. There was, however, a lack of an early increase in the DNA content, and an absence of an immediate rise in sucrase activity in the intestine of suckling rats. This suggested that the immediate adaptational response to intestinal resection is not as effective as in weaned rats. This does not, of course, preclude further adaptation occurring after the animal weans, and over a longer period of time before growth is completed.

SECTION TWO

THE INFLUENCE OF LUMINAL NUTRITION

CHAPTER FOUR

SHORT-TERM BYPASS

MUCOSAL MATURATION IN THE ABSENCE OF LUMINAL NUTRITION

The results of the first experiment suggest that intestinal resection in immature rats induces both an adaptational response and an early mucosal maturation in the intestine, and that the major effect was demonstrated downstream of the anastomosis. This downstream response may have been the result of the combined effect of both the increased luminal nutrition to the distal gut, brought about by removing part of the intervening intestine, and a systemic response. In many previous studies, however, luminal nutrition appears to have been the major stimulus to intestinal adaptation after small bowel resection (85, 381). Nevertheless, there was also an upstream response, and in other studies on mature animals and the human neonate it has been suggested that an upstream response represents the effects of a systemic stimulus (an enterotropic factor (385)), which in turn may be the result of the metabolic stress, induced either by the surgery itself (211) or the metabolic stress induced by fluid losses after intestinal resection (347, 380, 381, 382; see also Section 6.2 of the introduction). The limited upstream changes in this study may have been the result of a similar systemic response to stress (assuming that the infant rat does not become hyperphagic during the post-operative period - see Chapter 2 and refs. 119, 148). In an attempt to separate the two responses (the response to a systemic effect and the response to the direct effect of nutrients), mucosal maturation was studied in a bypassed loop of intestine in the immature suckling rat. Mucosal maturation could then be studied in isolation, in the absence of luminal nutrition in the bypassed segment. If

a systemic stimulus follows the stress of surgery or if an enterotropic factor emanates from the remaining in-line intestine, the effects of this stimulus should be observed in the bypassed segment.

In this experiment, mucosal maturation was examined by recording the changes in mitotic index, crypt depth, villus height and disaccharidase activities, and by examining cell migration and the concomitant maturation of enterocytes as they moved from crypt to villus tip.

METHODS

In this study, only 33% of the small bowel was removed from the nutrient stream, to ensure that animals grew normally and were not malnourished, as early malnutrition alters the pattern of mucosal maturation (127, 139, 277). Nevertheless, a 33% bypass was considered necessary, to ensure that there would be a systemic response (135, 137, 383), and also that there would be sufficient length of intestine from the proximal end of the bypassed loop to the ileo-caecal valve, to minimize any local response to reflux of nutrients into the loop.

Intestinal bypass of the last one third of the jejunum-ileum was carried out in 12 to 14 day old male Wistar rats ($n=14$), as shown in Fig. 4.1 (see also Fig. 5.1). When bypass is carried out in this manner, the self-emptying, isoperistaltic bypassed loop has an intact ileo-caecal valve, to minimize reflux of colonic contents, and possibly undigested nutrients, into the bypassed loop. Animal preparation was similar to experiment one. As in the first experiment, the anastomoses were carried out with interrupted 7.0 prolene, and the abdomen was closed with 6.0 silk. Control animals ($n=12$) underwent intestinal transection at the same point as the bypass started. The rat pups were returned to their dams after fully recovering from the anaesthetic, and they were seen to suckle almost straight away. They were also allowed free access to chow, and were seen to commence foraging at approximate-

ly 20 days of age. They were not separated from their dams at this age, as in the first experiment, as the last sacrifice point was at 22 days of age.

The mucosal response to intestinal bypass was assessed in one of four ways: 1) by the developmental profile of total mucosal enzyme activities in their chronological sequence; 2) by the assessment of the rate of accumulation of enzyme activity in the enterocyte as it migrated from the crypt to the villus tip; 3) by the assessment of the cell migration rate as the enterocytes migrated from the crypt up the villus; and 4) by histological examination.

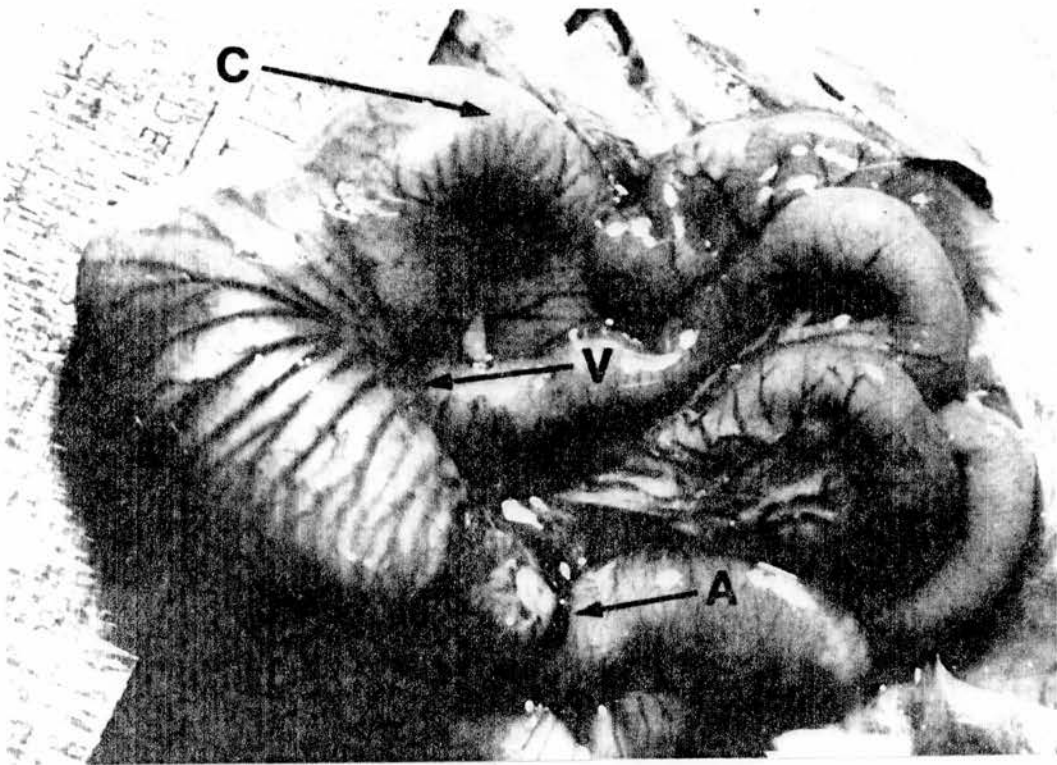


Fig. 4.1 The bypassed ileum at the time of sacrifice. A = the anastomosis between proximal ileum and caecum, V = the ileo-caecal valve where the bypassed ileum enters the caecum, and C = the colon.

Both 2) and 3) rely on a cell fractionation technique whereby the villi are gently shaken in a solution of EDTA in such a way that the villus gradually disintegrates sequentially from its tip down to the crypt (288, 373; Fig. 4.2). As the villus disintegrates progressively, cells are also released sequentially from villus tip down to and including the crypt base. (See also Appendix Two).

To demonstrate the chronological developmental profiles for lactase and sucrase activities, rats undergoing intestinal bypass or transection were sacrificed at 16, 17, 19, 20 and 21 days of age. Cell fractionation studies to determine the rate of enzyme accumulation within the enterocytes were carried out at 16 and 21 days of age, and cell migration studies were carried out in animals of 16 and 22 days of age. Two normal animals were sacrificed at 12 days of age, to provide "zero" points for the study. To determine the chronological sequence of enzyme changes as the mucosa matured, disaccharidase activity was assayed in whole gut, as mucosal scraping produced inconsistent results in these young animals.

After intestinal bypass, total disaccharidase activity was assayed in 1.0 cm specimens of the whole intestine taken, first from the most distal 1.0 cm of the ileum in continuity (Fig. 4.3)., and second from the most proximal 1.0 cm of the bypassed intestine. After intestinal transection, disaccharidase activities were assayed in the first 1.0 cm distal to the transection.

For the cell fractionation studies, after intestinal bypass, a 5.0 cm section was removed from 0-5 cms beyond the ligament of Treitz, and a further 5.0 cms was removed from 2-7 cms from the proximal point of the bypassed loop (Fig. 4.3). After intestinal transection, a 5.0 cm section was taken from 0-5 cms from the ligament of Treitz, and another 5.0 cms was taken from 2-7 cms distal to the transection. As each cell fraction was released by the shake-down process, enzyme activity was assayed in that fraction (268). As the enzyme activity on the brush border membrane of the

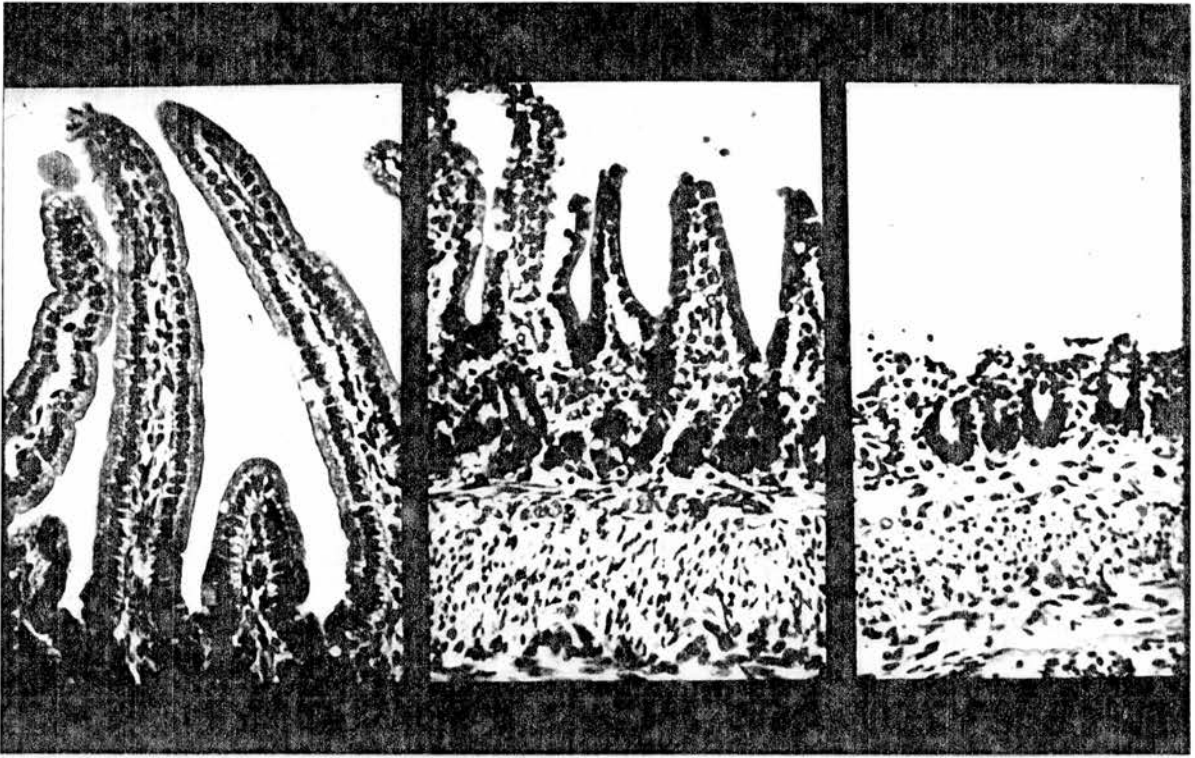
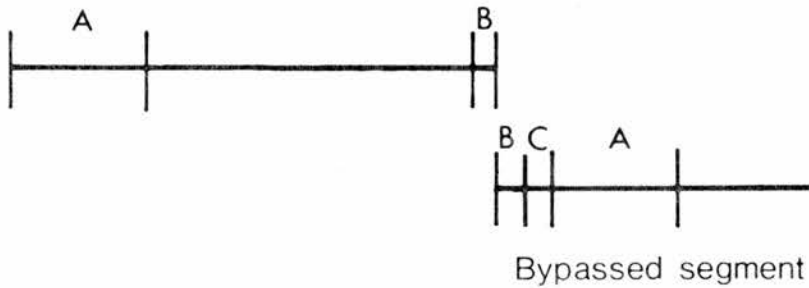


Fig. 4.2 Progressive removal of the cells of the villi from tip to crypt base using the "shake-down" technique.

EXPERIMENTAL INTESTINAL SEGMENTS

SEGMENT IN CONTINUITY



CONTROL INTESTINAL SEGMENTS

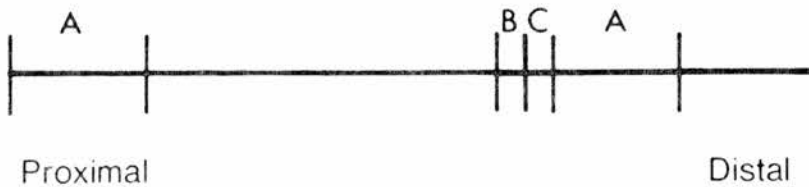


Fig. 4.3 Intestinal segments used. A = for cell fractionation studies, B = for total disaccharidase activity, C = for histology.

enterocyte normally increases as the cells move from crypt to villus tip, in this way a profile of the cellular enzyme activity was obtained as cells migrated.

The cell fractionation technique, as described above, was also used to estimate the rate of enterocyte migration (268). Twenty-four hours after the injection of tritiated thymidine, the average distance travelled by the enterocytes was determined by demonstrating which cell fraction contained the advancing wave of isotope.

The estimations of the disaccharidase activities were as in experiment one (see Appendix 1), except that activity was expressed per mg of protein for this experiment alone, and the developmental profile of lactase activity was measured as cellobiase activity, a more accurate determinant of brush border lactase activity (352, 353).

For histological examination, the second 1.0 cm of the bypassed ileum or the second 1.0 cm of the intestine distal to the transection was taken. The preparation of the specimens for histology was carried out as in experiment one.

RESULTS

The bypassed ileum became markedly narrowed after only six days (Fig. 4.1), with no gross evidence of reflux into the loop. All of the animals gained weight whether they underwent intestinal transection or bypass.

DISACCHARIDASES

When the intestine was excluded from the nutrient stream, lactase activity remained high (Fig. 4.4a) well beyond the age at which lactase activity normally declines (147, 288, 339). On the other hand, lactase activity declined normally in the

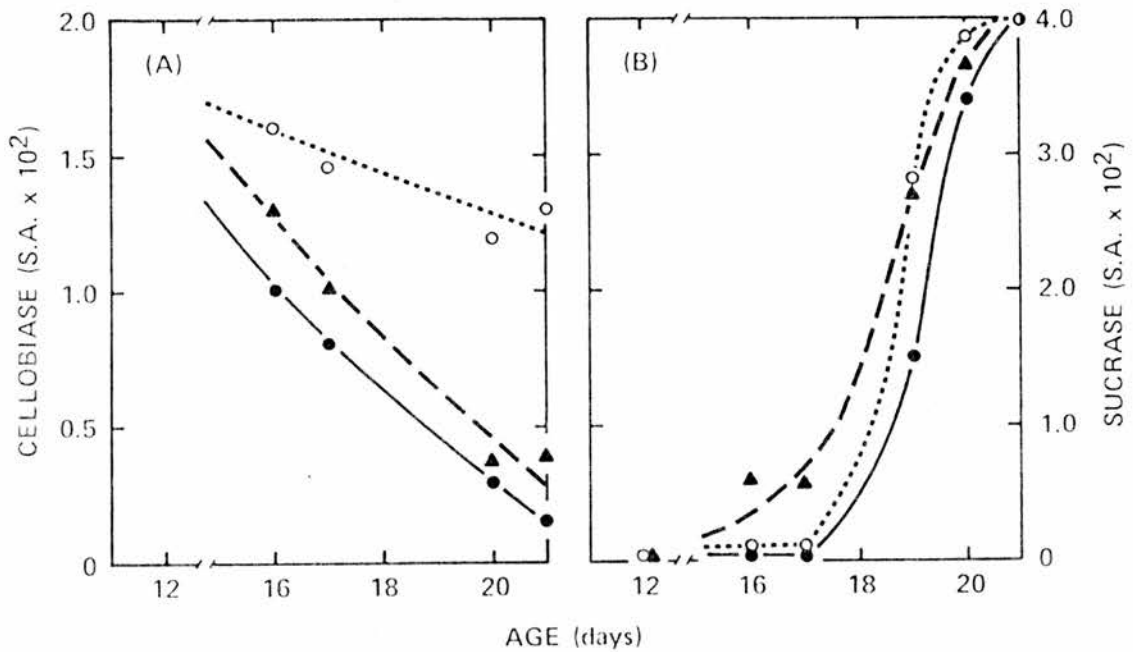


Fig. 4.4 Developmental patterns of (a) lactase (cellobiase), and (b) sucrase in the bypassed ileum, in-line ileum of the same rats, or normal ileum of the suckling rat. Dotted line with open circles represents the bypassed segments, dotted line with solid triangles represents the in-line segments, and solid line with solid circles represents littermate controls undergoing intestinal transection.

in-line jejunum of the same animals where the mucosa was in contact with the nutrient stream (Fig. 4.4a), and normally throughout the intestine of control animals. Sucrase activity, however, rose precociously in the proximal in-line intestine after distal intestinal bypass (Fig. 4.4b), but did not increase earlier than usual in the bypassed loop itself. Therefore, there appears to a paradox whereby in-line intestine is undergoing normal mucosal maturation, whereas in the bypassed intestine, in the same animals, maturation is delayed.

INCREASING DISACCHARIDASE ACCUMULATION DURING CELL MIGRATION

When jejunal disaccharidase activities and their rate of accumulation were examined in 16 day old rats in the cell fractions taken from the crypt to the villus tip, sucrase activity was present in the newly formed cells at the crypt-villus junction, but only in the 16 day old rat that had undergone intestinal bypass. Sucrase activity was not present in the older cells at the tip of the villus, and was not present in the dividing cells of the crypt (Fig. 4.5). In control (transected) animals, there was no sucrase activity in the jejunum or the ileum, in distinct contrast to the activity in the jejunum of the experimental (bypassed) rats. In the bypassed ileum of the experimental animal, however,

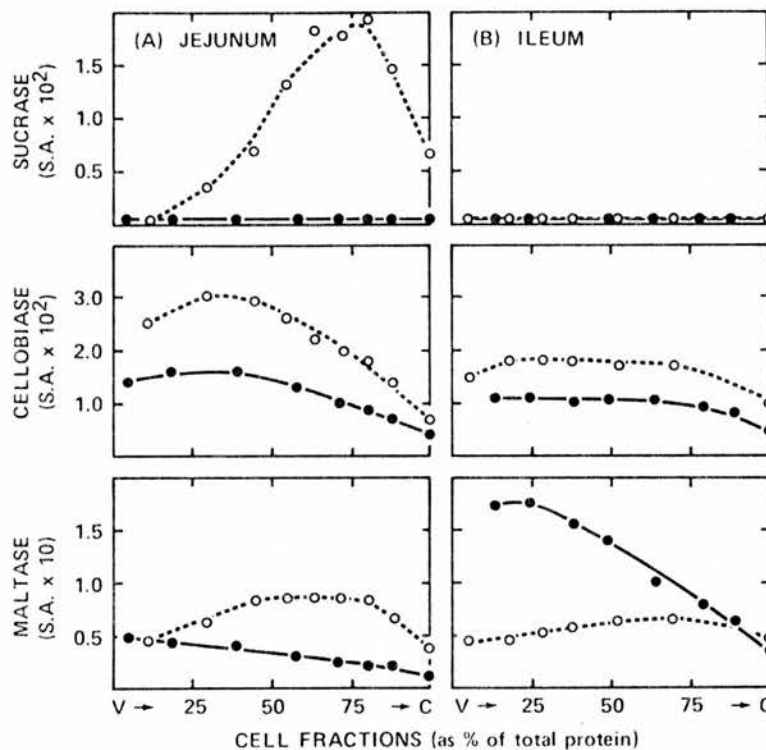


Fig. 4.5 Patterns of disaccharidase distribution across the crypt to villus unit in (A) jejunal and (B) ileal segments of 16 day old experimental and control suckling rats after intestinal bypass or intestinal transection at 12 days of age. The isolated cell fractions (see Methods) were assayed for disaccharidase activity and protein content. Key as in Fig. 4.4.

sucrase was also absent. Therefore, these results confirm in more detail that an apparent paradox exists where precocious maturation of sucrase activity can occur in one part of the intestine of an experimental animal, whereas in another part of the intestine of the same animal normal mucosal maturation of sucrase activity is seen. In contrast to the early appearance of sucrase activity, the accumulation of lactase activity was essentially normal in the 16 day old experimental animals, although there was a modest rise in lactase activity in both the jejunum and the ileum (Fig. 4.5). Maltase activity essentially followed the same developmental pattern as the sucrase activity, although maltase activity was present in the distal intestine in both control and experimental 16 day old rats.

At 21 days of age, the pattern of increasing concentration of disaccharidase activity in the enterocytes of the bypassed intestine was quite different from that in the in-line intestine (Fig. 4.6). Lactase activity was now increased in the bypassed ileum, but was similar in the jejunum of both control and experimental rats. Sucrase activity, on the other hand, was increased in the in-line jejunum after bypass, but was normal in the bypassed loop of ileum (Fig. 4.6). The pattern of accumulation of maltase activity was essentially the same as that of sucrase (Fig. 4.7). Again, there was a disassociation of the appearance of sucrase activity in one part of the intestine from the appearance of the same enzyme in another part of the intestine, and the opposite response for the disappearance of lactase activity was noted.

CELL MIGRATION STUDIES

Intestinal bypass prevented the normal cytokinetic changes that occur in the jejunum and the ileum at weaning (Fig. 4.7a&b). By injecting tritiated thymidine 24 hours before sacrifice, the average distance travelled by the enterocyte in the subsequent 24 hours can be estimated by the cell fractionation studies, as only the cells in the crypt have thymidine kinase and can take up thymidine and incorporate it into DNA (5). Therefore, using

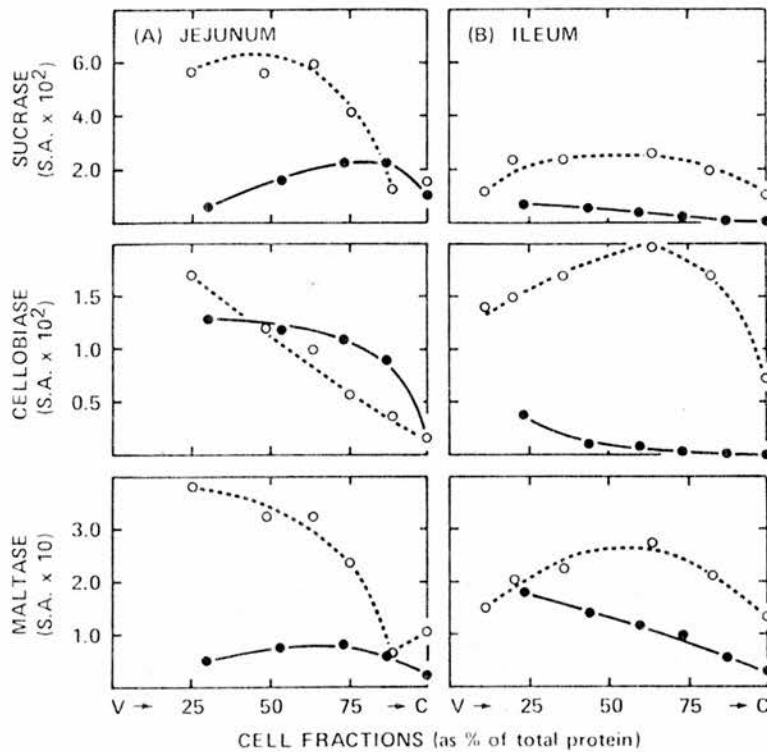


Fig. 4.6 Patterns of disaccharidase distribution across the crypt villus unit in (A) jejunal and (B) ileal segments of 21 day old animals undergoing intestinal bypass or transection at 12 days of age. Key as in Fig. 4.4.

this method, the cells with radioactive thymidine in their nuclei are detected as they migrate along the villus, and the fraction in which they are detected indicates how far they have migrated since the injection (see also Appendix 2).

In the jejunum of 22 day old rats, cell migration from crypt to villus tip was almost complete within the 24 hours after the injection of thymidine, and almost identical in control animals and those undergoing bypass (the cells near the villus tip contain radioactivity), whereas the cells in the jejunum and ileum of the 12 day old rats have hardly migrated at all (the cells close to the crypt contain the radioactivity).

After bypass, however, cells in the bypassed ileum of 22 day old rats migrate, as the cells in the ileum of a 12 day old rat,

i.e. slowly. Therefore, as the villus height in the ileum of the experimental and the control animals was similar, this lack of progress must represent a reduced migration rate.

HISTOLOGY

In the bypassed intestine, the villi did not demonstrate any atrophy in the six day study period, but crypt cell proliferation, as indicated by the numbers of cells undergoing mitoses, had decreased (Table 1).

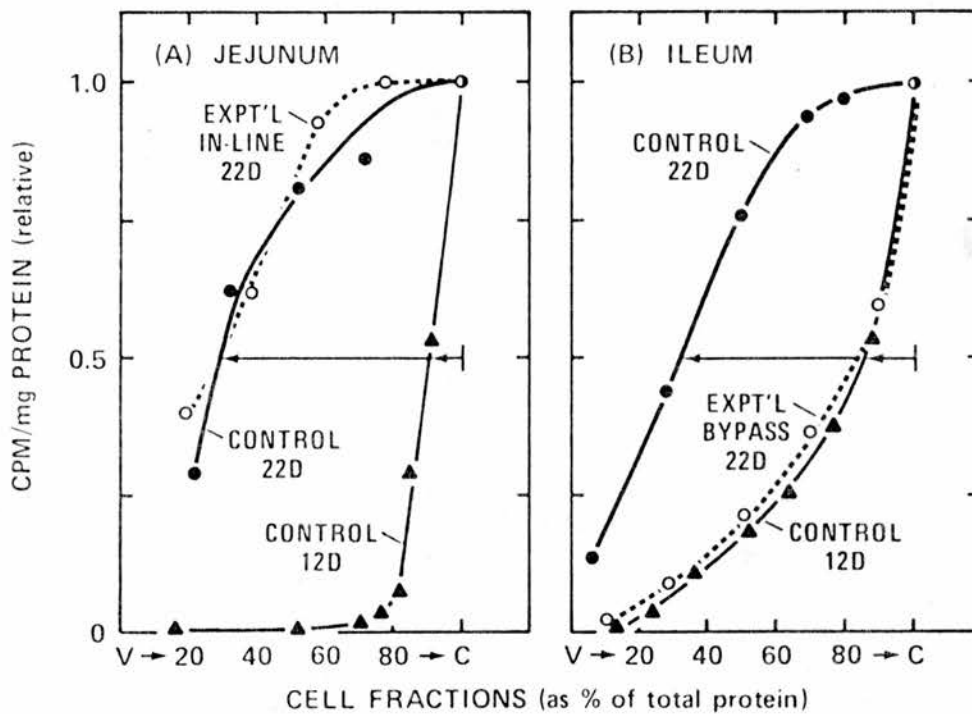


Fig. 4.7 Effect of surgical bypass on migration of epithelial cells in (A) jejunum of the shortened gut maintained in continuity and (B) bypassed ileum. Jejunal and ileal segments were removed from experimental and control animals 24 hours after a single injection of (3-H)-thymidine after intestinal bypass or transection at 12 days of age. Intestine was removed from the experimental animal at 22 days of age, and from the control animals at 12 and at 22 days of age for these cell migration studies (see Methods). Key as in Fig. 4.4.

Table 1 Measurements of villus height, crypt depth and mitotic cells in bypassed and control ileal segments in the suckled rat.

Measurements	Bypassed ileum ^a (n = 8)	Control (Transected) ileum ^a (n = 6)	Statistical significance (p)
Villus height (mm) ^b	0.26 ± 0.20	0.31 ± 0.03	NS
Crypt depth (mm) ^b	0.29 ± 0.01	0.12 ± 0.01	<0.05
Mitoses ^c	0.39 ± 0.08	0.78 ± 0.17	<0.05

^aRats were subjected to experimental bypass and transection (controls) at 14 days of age and intestinal segments removed for analyses 6 days later. Experimental and control animal weights did not differ significantly at the time of death.

^bMeasurements were performed on the 10 largest villus-crypt units and the average value ±SEM recorded.

^cAverage number of mitotic cells per half crypt (total of 10 half-crypt units examined). A "half crypt" was defined as that portion of the crypt continuous with the villus and extending from the crypt opening to the midline of the crypt base.

DISCUSSION

Bypass inhibits the normal acceleration of crypt cell migration and crypt cell proliferation that occurs in the ileum at the time of weaning. What is more, bypass also inhibits the normal fall in lactase activity in the bypassed segment, and induces an early rise in the sucrase activity in the proximal non-bypassed gut.

One of the advantages of the intestinal bypass model is that the presence of luminal nutrition can be studied in one and the same animal as the absence of luminal nutrition. In isograft studies, the direct effect of luminal nutrition on the immature intestine cannot be assessed, and the systemic response of the host to intestinal resection would represent the adult animal's

response to intestinal resection, not the response of the immature rat.

In previous studies with isografts of foetal mouse intestine, transplanted under the renal capsule of an adult rat, sucrase activity appeared on time, but lactase activity was still detectable well beyond the time at which it should normally have disappeared from the intestine (105). This would suggest that luminal nutrition is not necessary for the appearance of sucrase activity, but that luminal nutrition is in some way responsible for the disappearance of lactase activity. This is not merely the result of starvation brought about by intestinal surgery, because, when suckling rats are chronically starved from birth, the appearance of sucrase activity is delayed rather than accelerated, but the disappearance of lactase activity is also slowed (127, 139, 277, 320). Therefore, chronic starvation after the intestinal surgery cannot explain all of these results. Furthermore, when rats are weaned early, at 16 days of age, sucrase activity appears early, and this response appears to be dependent upon the adrenal response to the stress, as adrenalectomy abolishes the response (43). Furthermore, other generalized systemic disturbances can also alter the time at which sucrase activity appears, as systemic stimuli (211), as well as the hormonal status, can result in precocious mucosal maturation (147, 255). Therefore, it appears that sucrase activity will appear in immature rat intestine that receives nutrients only via the blood stream, whether that intestine is an isograft of foetal intestine or a bypassed loop, and the time of that event is apparently modified by the hormonal status of the animal and whether or not the animal is undergoing stress. Therefore, carbohydrate does not apparently need to be present within the intestinal lumen for sucrase activity to appear in the intestine of the weaning rat, but the presence or the absence of carbohydrate at this sensitive time in intestinal development (147) appears to "fine tune" the rate of appearance of this enzyme. Moreover, sucrase does not have to be the responseible carbohydrate for sucrase induction, as intestinal sucrase activity can also be induced in weanling rats by placing them on a high lactose diet

(149), and breast milk contains a high concentration of lactose. Lactase, however, is not an inducible enzyme, and its disappearance seems to be modified by stress and thyroxine, rather than stress and corticosteroids (255, 359).

Thus, these studies show that lactase activity will remain high in a situation where luminal nutrition is absent, but where systemic nutrition is normal (rats grew normally after both intestinal bypass and transection). Therefore, the absence of a fall in lactase activity is also not the result of chronic systemic malnutrition, but appears to be specifically related to the absence of luminal nutrition.

The absence of an early decline in lactase activity, and the absence of an early rise of sucrase activity in the bypassed segment would seem to suggest that, after intestinal bypass at least, there was no systemic response stimulating the bypassed loop to mature early. Quite the contrary, if a systemic stimulus was present, it was not influencing mucosal enzyme activity in the bypassed loop. Of course, this could merely be a reflection of only 33% of the intestine being removed from the intestinal stream, and not 60%, as in the initial experiment. There was, however, a definitive rise in sucrase activity in the proximal in-line jejunum in the bypassed animals, suggesting that some systemic response was indeed present, and was stimulating the proximal gut. This response is unlikely to be explained on the basis of increased food intake after the loss of absorptive surface area, as occurs in older animals (250), as these suckling pups lack the central controls necessary to increase their food intake while nutritionally stressed (147). One explanation of the lack of response in the bypassed loop at the same time as a response occurred in the proximal in-line jejunum may be that an enzyme's substrate has to be present within the lumen of the intestine before any systemic effect can be expressed, and both the substrate and the stress have to be present to demonstrate the systemic response, *i.e.* the substrate acts in a permissive role for the systemic effect to occur. On the other hand, these results may merely reflect the fact that

the jejunum is apparently more sensitive to an enterotropic factor than the ileum (137, 383).

Another advantage of this animal model is the ability to monitor the changes in cell migration that occur at the beginning of the weaning period in normal intestine, in in-line intestine after part of the absorptive surface area has been removed, and in bypassed intestine. These studies would be difficult, if not impossible, to carry out in isografts of foetal intestine. In this study, the absence of the abrupt acceleration in cell migration in bypassed intestine must have been due to the lack of luminal nutrition, as the animals were otherwise growing normally, and a normal abrupt increase in cell migration occurred in the jejunum at the same time, and a similar abrupt increase in cell migration occurred in the ileum of control rats. This lack of an abrupt acceleration of cell migration rates to similar values to those in adult animals also serves to underline a further basic immaturity in the cells of the bypassed intestine, apart from the delayed mucosal enzyme changes. One further observation in this animal model is that cell migration was not faster than normal in the proximal in-line intestine of the experimental animals, despite such observations in residual intestine after partial enterectomy in mature rats (381), and despite the effect of whatever stimulus induced an early appearance of sucrase activity in the same segment of the proximal gut. Therefore, as in the first experiment, weaning appears to interfere with, or even block, the rat's ability to mount a cytokinetic adaptational response to the loss of intestinal absorptive surface area. Possibly the acceleration of cell migration that normally occurs at weaning is so great that no further increments in cell migration were possible.

Furthermore, the number of mitoses were decreased in the crypts of the bypassed bowel, demonstrating diminished crypt cell proliferation. As a result, this might imply that prolonged periods of absent intraluminal nutrition during normal gut growth might lead to a permanent lack of growth in that intestinal segment. Therefore, this study would tend to suggest that the prolonged

absence of luminal nutrition could be detrimental in the clinical situation, where the human neonate is placed on long-term intravenous nutrition.

Nevertheless, this study did not allow for the effects that bile and pancreatic secretions may have had if they had been present in the bypassed loop. In the premature neonate, however, the flow of pancreatobiliary secretions is slow (see introduction, the section on development of normal digestion), and the flow of both is further reduced by parenteral nutrition (22, 24, 161, 278, 287, 350), especially in the premature (22). Therefore, the situation depicted in this study is not dissimilar to the clinical situation, where premature human neonates are placed on long-term parenteral nutrition after intestinal resection.

SUMMARY

This experiment has concentrated on the short-term response to intestinal bypass in the weaning rat as it begins to mature from a totally atricial animal to an omnivore. The study demonstrated that: there was no increased rate of enterocyte migration from crypt to villus tip in the gut that remained in continuity, despite the early appearance of sucrase in the same segment of bowel; lactase activity increased initially in the in-line intestine of the 16 day old rat, suggesting that there could be enzyme induction at this early age, but lactase activity decreased in the cells appearing at the base of the villus in the jejunum of the 21 day old rats that had undergone intestinal bypass, suggesting that by the age of 21 days stress can induce an early fall in lactase activity (similar to the fall in lactase activity 6 days after 60% small bowel resection in the 14 day old rat in the first study), and that in bypassed bowel lactase activity did not decline normally, and remained at high levels; sucrase will appear early in the intestine of the stressed animal, especially where intestinal absorptive surface area has been removed, but only in segments of the intestine that are exposed to the nutrient stream. The following chapter examines the longer term response to bypass.

CHAPTER FIVE

LONG TERM BYPASS

In the previous experiment on the short term effects of bypass, crypt cell proliferation reduced by approximately 50%, and cell migration was markedly inhibited, after only ten days. As longitudinal growth of the intestine is quite possibly the result of crypt fission (65, 247), and as bypass in older rats (307) and rabbits (190) produces reduction in the length of the bypassed segment, then bypass of a segment of intestine at an early age, when intestinal growth is especially rapid (198), could lead to profound longitudinal growth inhibition in the intestine, especially if the bypass was maintained for a longer period of time. In a similar situation in the neonatal human being, prolonged parenteral nutrition without any enteral nutrition after major gut surgery could inhibit both compensatory and normal intestinal growth.

Not only is longitudinal growth of the intestine inhibited after intestinal bypass, but mucosal growth is inhibited also. For example, after jejunal bypass in adult animals, when a small length of the jejunum was bypassed, the mucosa of the bypassed segment demonstrated a 30% decrease in the numbers of cells on the villi, and a 10% decrease in the number of cells in the crypts (297). At the same time, there was a 30% fall in DNA synthesis (297). This atrophy appears to be the result of removing the intestine from the nutrient stream (12, 85, 137, 381, 383, 384).

There are, however, differences in the degree of atrophy, depending on which part of the intestine is bypassed, and these differences emphasise the role of luminal nutrition. When the jejunum is bypassed, the nutrient stream would normally contain chyme rich in pancreatico-biliary secretions, and a high concentration of partly digested nutrients (12, 85, 384, 387). As a result, when this stimulus is withdrawn, the mucosa no longer maintains its normal rate of cell production and villus height. When a short segment of the ileum is bypassed, however, the response

is not the same as that seen when the jejunum is bypassed. The villi undergo no atrophy, the cells per crypt column do not decrease, the nucleic acid content does not fall, and DNA synthesis remains similar to that seen in the intestine of controls (137, 383). Furthermore, in the rabbit, the crypt, far from showing diminution in size, may actually increase in size by up to 60%, while the villi decrease in size by a similar amount after this procedure (190).

When a large portion of the intestine is removed from the nutrient stream, either by bypassing a long segment or by bypassing a short segment and resecting part of the remaining functioning gut, the gut that remains within the nutrient stream apparently releases a stimulatory (enterotropic) factor that can abolish the hypoplasia seen after jejunal bypass and, when the ileum is bypassed, can actually increase DNA content and produce modest hyperplasia in comparison with the normal ileum (137, 297, 383).

Despite the ileal hyperplasia seen after bypass of the ileum and resection of the in-line intestine, the response to this enterotropic factor is more marked in the jejunum than the ileum, for, although the jejunal response does not produce a mucosa that is hyperplastic in comparison with normal rat jejunum, hypoplasia after jejunal bypass is so marked that the return to normal structure represents a far greater response to an enterotropic factor than the minimal hyperplasia seen after bypass of the ileum (137, 383). Therefore, the jejunum of the rat appears to be far more sensitive to this enterotropic factor than the ileum.

As any bypass would remove intestine from the nutrient stream, enterotropic stimulation of the bypassed loop would occur. Therefore, to try to minimize this stimulus as far as possible to examine intestinal growth in a similar situation to where parenteral nutrition is being used, the ileum was used rather than the jejunum. Nevertheless, as the length of the loop bypassed increases, so the accuracy of recording any longitudinal growth responses also increases. Therefore, instead of bypassing 30% of intestine in this

study, 40% was bypassed, accepting that this would increase the potential for enterotropic stimulation of the mucosa of the bypassed loop.

Accepting these limitations, we assessed the relative contributions to the adaptational response to intestinal resection of 1) obligatory growth (growth that occurs in an organ that has no functional demand placed upon it (328)); 2) normal growth (growth that occurs in an organ that has a normal functional demand placed upon it); and 3) compensatory growth (growth that occurs in the residual part of an organ or organ system when an abnormal functional demand is placed upon it, *e.g.* after part has been removed). For this purpose, the distal 40% of the small bowel was bypassed, or the proximal 60% was removed. Control animals had simple intestinal transection. Therefore, bypassed intestine would have decreased functional demand, the residual intestine after intestinal resection would have an increased functional demand and, after intestinal transection, there would be normal functional demand.

METHODS

The animal model used for this experiment was essentially the same as that for the previous experiment except that, with more experience, this bypass procedure could be performed reliably on ten day old rats. The only additional procedure was the animal with the 60% proximal small bowel resection (Fig. 5.1). Animals were allowed to wean naturally, and were totally independent of their dams by 30 days of age. They were sacrificed four weeks after the bypass at 38 days of age.

For the nucleic acid determinations, the assays used were those of Scott, Burton and Hinrichs (58, 154, 322; also see Appendix One). Biochemical assays were carried out on mucosal scrapings in these older animals. For the estimations of the villus height, five complete villi and five complete crypts were counted for each specimen and from these the average villus height and crypt depth for that specimen was calculated.

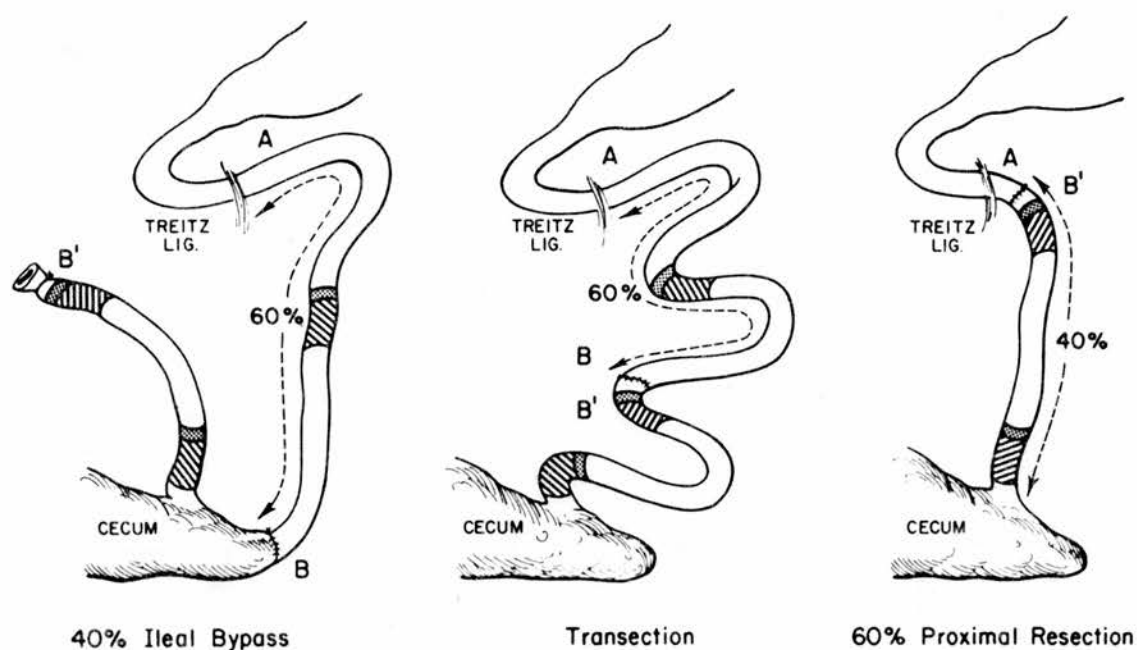


Fig. 5.1 The intestine is transected at B,B' (see middle figure) in all animals. When bypass of the ileum is to be carried out, B' is simply closed with a single ligature and B is anastomosed to the caecum at the site of the appendix stump after the appendix has been removed. When simple intestinal transection is carried out, B,B' is immediately re-anastomosed. When proximal intestinal resection is to be carried out, the segment A,B is resected and B' is anastomosed to A. Shaded areas represent the site of origin of specimens for histological examination, and the cross-hatched areas are the sites for nucleic acid estimation.

The length of the intestine was initially measured *in situ* at the time of bypass, while the animal was anaesthetized. The mean length of the intestine in all of the animals determined in this way was used as the base line value for intestinal length. Four weeks later, at sacrifice, the animals were exanguinated under ether anaesthesia, the gut was removed, the mesentry was stripped from it, and the intestinal lengths were then measured under 5 g

tension, and not the 2 g tension used for the younger animals. The intestinal circumference was measured after opening the proximal ileum longitudinally, and before storage of the specimens for biochemical assay.

RESULTS

Sixty-nine per cent of the pups (83/120) survived the operations, and suckled successfully to gain weight. Those animals undergoing intestinal transection were significantly heavier than those undergoing intestinal bypass or intestinal resection (Table 5.1). There was no difference in the weights of those animals undergoing intestinal resection or bypass. Because the length of the intestine increases with body weight in young rats (198), only animals weighing between 105 g and 135 g were studied further. This left a total of 45 animals to be studied (Table 5.2). There was no significant difference between the body weights when animals were selected in this manner.

Table 5.1

		Weight g \pm S.E.
Ten day old rats	n = 120	20.6 \pm 0.3
Bypass	n = 34	106.9 \pm 2.7 A
Transection	n = 23	125.9 \pm 3.0 B
Resection	n = 26	109.9 \pm 3.8

A = Bypass v Transection $p < .001$

B = Transection v Resection $p < .01$

Table 5.2

		Weight g \pm S.E.
Bypass	n = 18	116.9 \pm 1.2
Transection	n = 13	118.5 \pm 2.2
Resection	n = 14	116.4 \pm 1.8

After intestinal resection and transection, the distal 40% of the jejuno-ileum was longer than after bypass. It increased by 381% after resection, by 365% after transection, and by 291% after bypass ($p = <.001$ for all three - Fig. 5.2). Resection of the proximal bowel induced no greater elongation of the ileum than transection. After intestinal transection, the ileum increased 25% more than after bypass ($p = <.001$) and after proximal intestinal resection the ileum increased 31% more than after bypass ($p = <.001$). Nonetheless, the length of the bypassed ileum increased also during the four week period. After both intestinal bypass and transection, the jejunal length was similar.

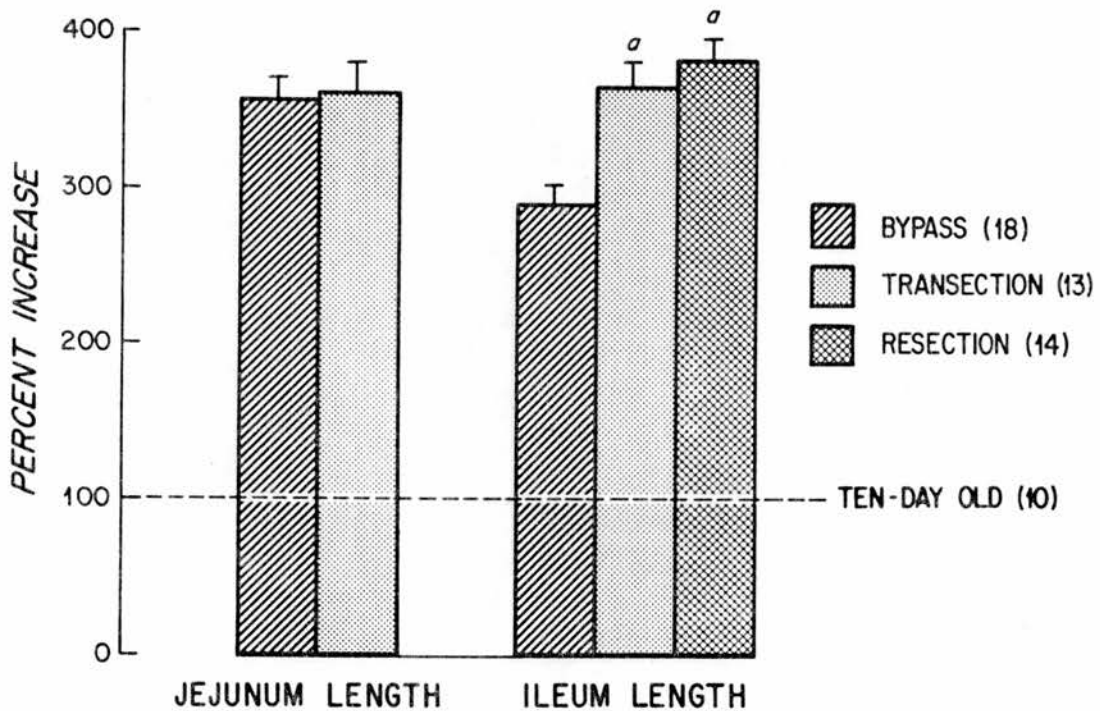


Fig. 5.2 Intestinal length at sacrifice expressed as a percentage increase from the time of surgery (\pm S.E.). The dotted line represents the mean length of the relevant intestinal segments measured in anaesthetized rats, in situ, at the time of surgery. "jejunum" = A,B in Fig. 5.1; "ileum" = B'-ileo-caecal valve in Fig. 5.1. A = $p < .001$ v bypass.

The circumference of bypassed ileum was static as compared with the values at 10 days of age but, after transection, the circumference increased by 112% ($p = <.001$) and, after proximal intestinal resection, the circumference increased by 162% ($p = <.001$) (Fig. 5.3). The ileal circumference after proximal resection was 23% greater than after intestinal transection ($p = <.02$).

In normal 10 day old rats and 38 day old rats after intestinal transection, there was an aboral gradient of villus height (Fig. 5.4). When the ileum was bypassed, the jejunal villi increased in height by 72% in four weeks ($p = <.001$) and, when the ileum was transected, the jejunal villi increased in height by 37% in the same period ($p = <.001$). The jejunal villi after bypass were 26% taller than after intestinal transection ($p = <.001$). To the contrary, the villi in the proximal ileum after bypass did not grow at all, but the villi in the proximal ileum after transection had grown by 19% ($p = <.01$), and after proximal resection by 81% ($p = <.001$). The villi after resection were 52% taller than after transection ($p = <.001$) and 72% taller than after bypass ($p = <.001$). In the distal ileum, the villi in the bypassed loop had grown by 53% ($p = <.001$), but not significantly after transection (bypass v transection $p = <.001$). After intestinal resection, the villi had grown by 43% ($p = <.001$) and were 7% shorter than after intestinal bypass (resection v bypass N.S.: resection v transection $p = <.001$).

In normal 10 day old rats, and in 38 day old rats, after intestinal transection, there was an aboral gradient of crypt depth (Fig. 5.4). After intestinal bypass, the crypts in the jejunum had elongated by 404% ($p = <.001$), and after intestinal transection had elongated to the same extent. In the proximal ileum after bypass the crypts had elongated by 291%, after transection by 331%, and after resection 404% ($p = <.001$ or all three v 10 day old). Resection produced a 17% greater crypt depth than transection ($p = <.05$), a 29% greater crypt depth than bypass ($p = <.001$), and transection produced a 10% greater crypt depth than bypass

PROXIMAL ILEUM CIRCUMFERENCE

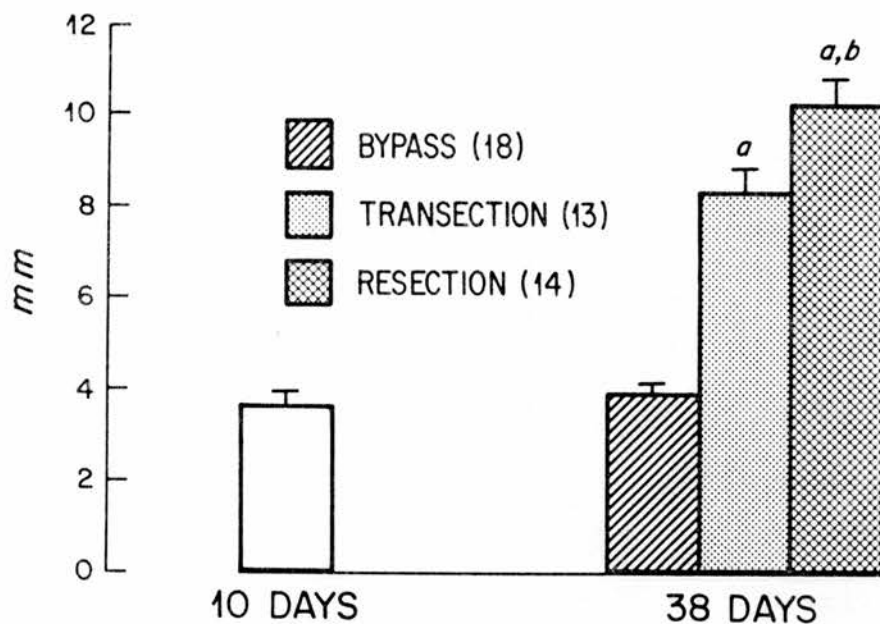


Fig. 5.3 The circumference of the proximal ileum at the time of sacrifice (\pm S.E.). A = $p < .001$ v bypass; B = $p < .02$ v intestinal transection.

(N.S.). In the distal ileum, the crypt had elongated by 373% after bypass, by 386% after transection, and by 476% after resection ($p = <.001$ for all three). The difference between bypass and transection was 3% (N.S.), between bypass and resection was 22% ($p = <.001$), and between resection and transection was 18% ($p = <.05$).

In normal 10 day old rats, and 38 day old rats, undergoing intestinal transection, there was an aboral gradient of intestinal DNA content (Fig. 5.5). Four weeks after intestinal bypass, or transection, the DNA contents of the jejunum were almost identical. The increase after bypass was 300%, and after transection was 319% ($p = <.001$ for both, transection v bypass N.S.). In the proximal bypassed ileum, the DNA content increased by 73% after bypass, by 364% after transection, and by 517% after resection ($p = <.001$ for all three v 10 day old; transection v bypass

$\rho = <.001$; transection v resection $\rho = <.001$; and resection v bypass $\rho = <.001$). In the distal ileum, the DNA content rose by 225% after bypass, by 560% after transection, and by 507% after resection ($\rho = <.001$ for all three; transection v bypass $\rho = <.001$; resection v bypass $\rho = <.001$; and transection v resection N.S.).

In normal 10 day old rats and 38 day old rats after intestinal transection there was an aboral gradient of the intestinal RNA content (Fig. 5.6). Four weeks after intestinal bypass, or transection, the RNA contents of the jejunum were almost identi-

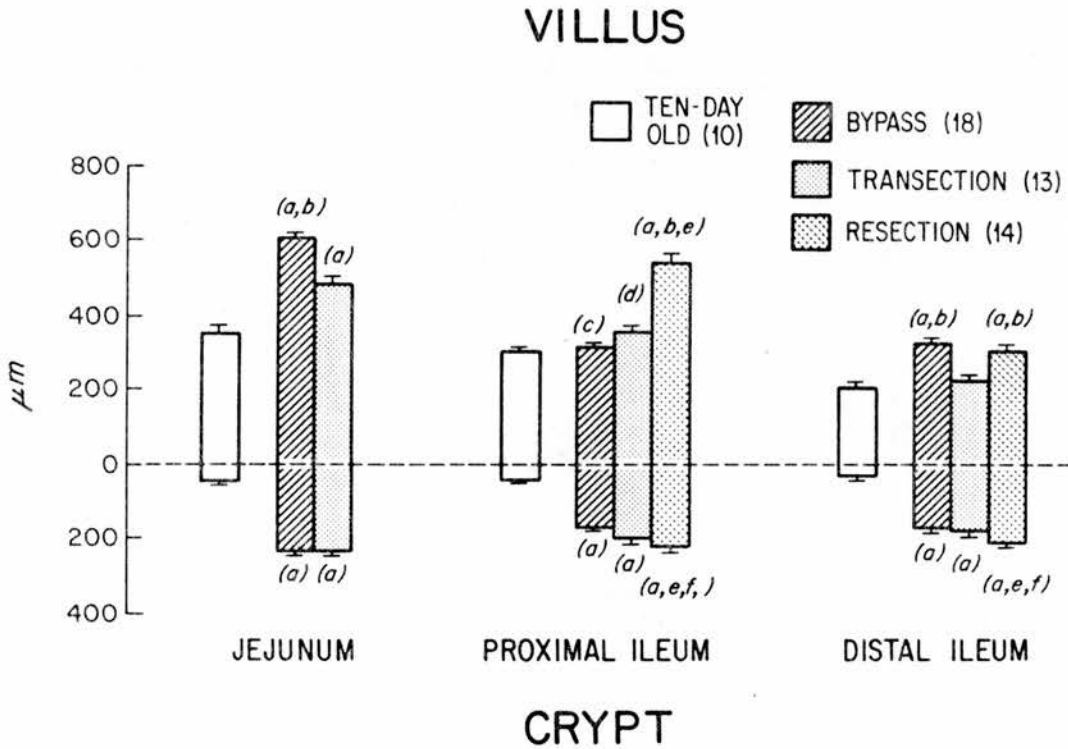


Fig. 5.4 Villus height and crypt depth (\pm S.E.) in the jejunum, proximal ileum, and distal ileum (shaded areas in Fig. 5.1). A = $\rho < .001$ v 10 day old rats; B = $\rho < .001$ v intestinal transection; C = $\rho < .02$ v intestinal transection; D = $\rho < .01$ v 10 day old rats; E = $\rho < .001$ v bypass; and F = $\rho < .05$ v intestinal transection.

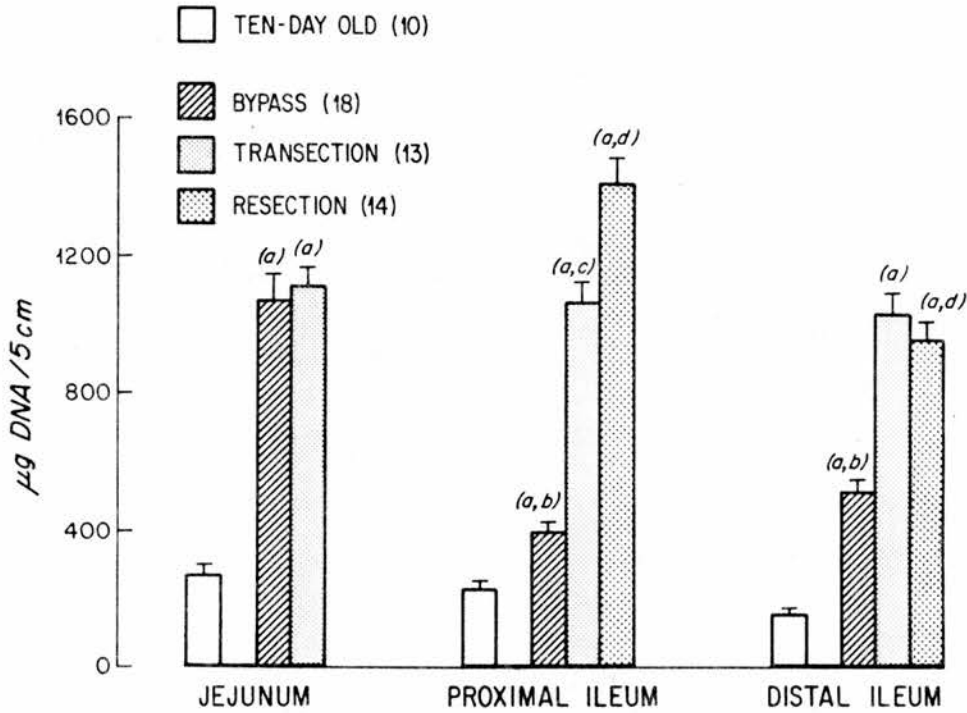


Fig. 5.5 DNA content of 5 cm sections (\pm S.E.) of the jejunum, proximal ileum, and distal ileum (cross-hatched areas in Fig. 5.1). A = $\rho < .001$ v 10 day old rats; B = $\rho < .001$ v intestinal transection; C = $\rho < .001$ v resection; and D = $\rho < .001$ v bypass.

cal. It had increased by 220% after bypass, and by 216% after transection (bypass and transection v ten day old $\rho = < .001$). In the proximal bypassed ileum, the RNA content rose by 6% (N.S.), by 103% after transection, and by 127% after resection ($\rho = < .001$ for all three; bypass v transection $\rho = < .001$; bypass v resection $\rho = < .001$; resection v transection $\rho = < .001$).

DISCUSSION

Nutrition must be present within the lumen of the intestine after intestinal resection for maximum intestinal elongation and maximum mucosal growth. Therefore, this study suggests that par-enteral nutrition alone, after intestinal resection, could be detrimental to the human neonate.

Previous experiments have shown that a similar obligatory growth will occur in other organ systems in the body when there is no functional demand placed upon that organ. For instance, when the kidney of an infant rat is transplanted into a syngeneic adult rat, renal growth occurs even when both of the kidneys of

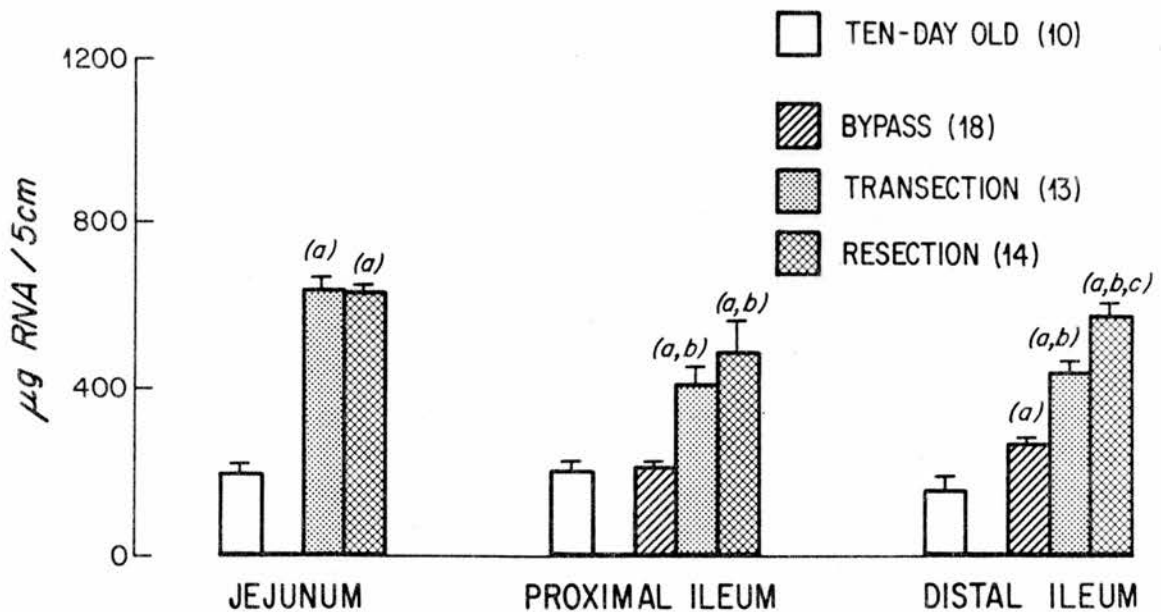


Fig. 5.6 RNA content of 5 cm segments (\pm S.E.) of the jejunum, proximal ileum, and distal ileum (cross-hatched areas in Fig. 5.1). A = $p < .001$ v 10 day old rats; B = $p < .001$ v bypass; and C = $p < .01$ v intestinal transection.

the adult rat are functioning normally (163, 328). This would suggest that infant organs will grow regardless of the demand placed upon them but, nevertheless, some compensatory growth over and above normal growth will occur when part of that organ system is removed (163, 328).

Despite the absence of luminal nutrition, basal (or obligatory) longitudinal growth occurred, so that the intestine almost tripled in length over four weeks. But, when a normal functional demand was placed upon the intestine, the intestine became almost four times its original length in that four week period. Excess functional demand, however, did not produce further growth. Therefore, as previously suggested (295, 296), observations as to the existence of compensatory intestinal elongation over and above normal growth after intestinal resection in the human neonate must be placed in serious doubt. Nevertheless, such observations are occasionally recorded (182).

Mucosal growth did not follow this all or none response to luminal nutrition, as a more linear response occurred which followed the increments in luminal nutrition *pari passu*. Furthermore, the mucosal response was not the same in the crypt and the villus compartments. The proliferative compartment (the crypt) demonstrated an almost threefold increase in size in the absence of luminal nutrition, while the functional compartment (the villus) failed to grow at all in the absence of luminal nutrition. There are several possible explanations as to why the crypt should grow while the villus did not. Firstly, during the physiological changes that normally occur at weaning, the crypt size changes abruptly (151). Therefore, the response in the crypt may reflect no more than the usual, possibly programmed, increase in crypt size that should occur at weaning (199, 243). The initial short term bypass study would tend to refute this explanation, as the numbers of mitoses in the crypt diminished in the bypassed segment in only 10 days. But, the delay in the mucosal maturation recorded in the short term bypass experiment does not necessarily imply that the redifferentiation of the intestine would be delayed indefinitely.

ly. Another possible explanation, however, could be that the increase in the crypt size in the absence of luminal nutrition could be the result of the effects of an enterotropic hormone stimulating the crypts to grow (2, 6, 34, 137, 172, 190, 383). If this were the case, and crypt fission is the mechanism whereby the intestine elongates, then the "obligatory" growth observed may have been the result of this enterotropic growth factor, and not basal or obligatory growth at all; or, at least, the basal growth would have been less marked if this factor was not at work. Nevertheless, when 90% small intestinal bypass is carried out in the mature animal, no such elongation occurs in the bypassed segment, despite an 80% increase in the length of the functioning gut (307). Therefore, it seems unlikely that an enterotropic factor could produce the intestinal elongation recorded here, or the increased crypt depth. One further explanation of the increased crypt depth in the absence of luminal nutrition might be that, as the villi did not increase in length to the same extent as the villi in the proximal ileum of the control animals, a local inhibitory influence does not come into play when the villi are short and, as a result, crypt cell proliferation is unchecked (20, 298).

The absence of villus growth in the bypassed segment stands out in distinct contrast to the longitudinal growth and crypt growth. This is unlikely to represent mere atrophy obscuring an underlying obligatory growth, as the villi in the ileum do not normally atrophy markedly after bypass in rats (137, 383). Therefore, it appears that the functional element of the intestine will not grow without a direct stimulus.

In the suckling animal, a special situation exists where their food, breast milk, contains mitogens that may stimulate the intestinal epithelium to grow (62, 194, 195, 342). Therefore, the absence of these mitogens from the bypassed loop could also explain the reduced intestinal growth.

This experiment implies that growth of the intestine could be permanently inhibited by the prolonged absence of luminal nutrition, especially in the more relevant clinical situation, where there would be no nutrients anywhere in the gastrointestinal tract to stimulate the release of an enterotropic factor, even if it does have a role to play. Therefore, in the next experiment, the reversibility of this growth inhibition was studied, by placing growing rats on parenteral nutrition, after 70% intestinal resection. When intestinal growth inhibition had been demonstrated in half of the rats, the others were allowed to feed normally for the subsequent four weeks to see if catch-up growth could be achieved.

CHAPTER SIX

PARENTERAL NUTRITION

In mature animals, the adaptational response to an intestinal resection is inhibited by parenteral nutrition (101). In the immature animal, both normal growth of the intestine and the adaptational response to resection are inhibited by intravenous feeding (196, 257, 258). Therefore, the therapeutic tool utilized to ensure immediate survival after massive intestinal resection in human neonates may well suppress the intestinal response to that resection, and perpetuate the need for intravenous feeding and its dangers.

Nevertheless, parenteral nutrition has become a common therapeutic tool that is life saving for many human neonates undergoing massive intestinal resection (140). There are, however, many potential dangers in its usage. Apart from the commonly encountered complications of sepsis, line blockage, line displacement, and venous thrombosis (140), there are also the problems of cholesta-

sis, cholelithiasis, and liver fibrosis (22, 24, 27, 191), in addition to the potential dangers of intestinal growth inhibition.

In the last experiment, the inhibition of the longitudinal growth brought about by four weeks of intestinal bypass suggests that absence of luminal nutrition for any length of time might lead to a permanent inhibition of longitudinal intestinal growth. Furthermore, in the absence of luminal nutrition, the inhibition of mucosal growth might also lead to a permanent inhibition of the adaptational response to intestinal resection. Indeed, when human neonates are placed on parenteral nutrition after massive intestinal resection, functional adaptation may take up to six months (126), despite the observation of greater than normal mucosal growth during this period of adaptation (182). Although this six month period might merely represent the time it takes for normal growth to occur in the residual in-line intestine, so that sufficient absorptive surface area for enteral support is provided (296), the parenteral nutrition itself may also prolong the period of adaptation.

In the bypass study, part of the residual growth observed in the bypassed loop may have been the result of the release of an enterotropic factor from the in-line intestine, which stimulates DNA synthesis and mucosal hyperplasia in bypassed gut (2, 137, 383, 389). Therefore, if the animals had been fed exclusively by vein, the inhibition of intestinal growth might have been more severe when there were no nutrients elsewhere in the gastrointestinal tract. Therefore, these observations suggest that there exists the potential for marked, and even permanent, inhibition of normal and compensatory growth within the residual intestine when human neonates are fed by vein after intestinal resection.

To test this hypothesis, immature rats were subjected to a 70% mid-small bowel resection, and then fed by one of three different methods for the subsequent ten days. One group was fed with an intravenous solution by vein, a second group was fed with the same intravenous solution by mouth, and a third group was al-

lowed to feed normally, with free access to laboratory rat chow. The subsequent inhibition of adaptation was examined in approximately half of the animals after ten days, while the reversibility of this inhibition was studied in the others after a further four weeks of normal feeding.

MATERIALS AND METHODS

Seven week old immature male Wistar rats, 90-95 grams ($n=163$) were housed for ten days prior to surgery in wire-bottomed cages, in an isothermic, light cycled, environment. Thirty-three of these rats were used as controls, and were fed rat chow throughout.

All rats subjected to intestinal resection ($n=130$) were starved for twelve hours before surgery, which in turn was performed under light ether anaesthesia. Seventy per cent of the jejunum-ileum was removed, leaving 15% of the jejunum-ileum proximally and distally. End-to-end anastomosis was carried out with 6.0 silk. Twelve normal rats were sacrificed under ether anaesthesia at seven weeks of age, and the small bowel was removed and sampled, as described below.

Fifty rats were fed via a central vein (216, 336; Fig. 6.1), and the remaining 80 rats were fed by mouth. For those fed by vein, a fine bore silastic catheter (0.012 inch I.D., Dow Corning) was placed in the internal jugular vein, and a modified amino acid-dextrose solution was administered (216). The dextrose concentration was reduced from the 30% normally used in older animals (216) down to 20%, to prevent line blockage. The daily volume delivered to each rat was 200 ml/kg/day for the first 24 hours after surgery, and 400 ml/kg/day for the subsequent 9 days. The solution also contained sufficient gentamicin to provide 3 mg/kg/day to each rat. Rats were allowed free access to water.

Rats that were fed by mouth ($n=80$) were equally divided into two feeding groups for ten days. 12 hours after surgery, one group was allowed free access to rat chow and water, and the

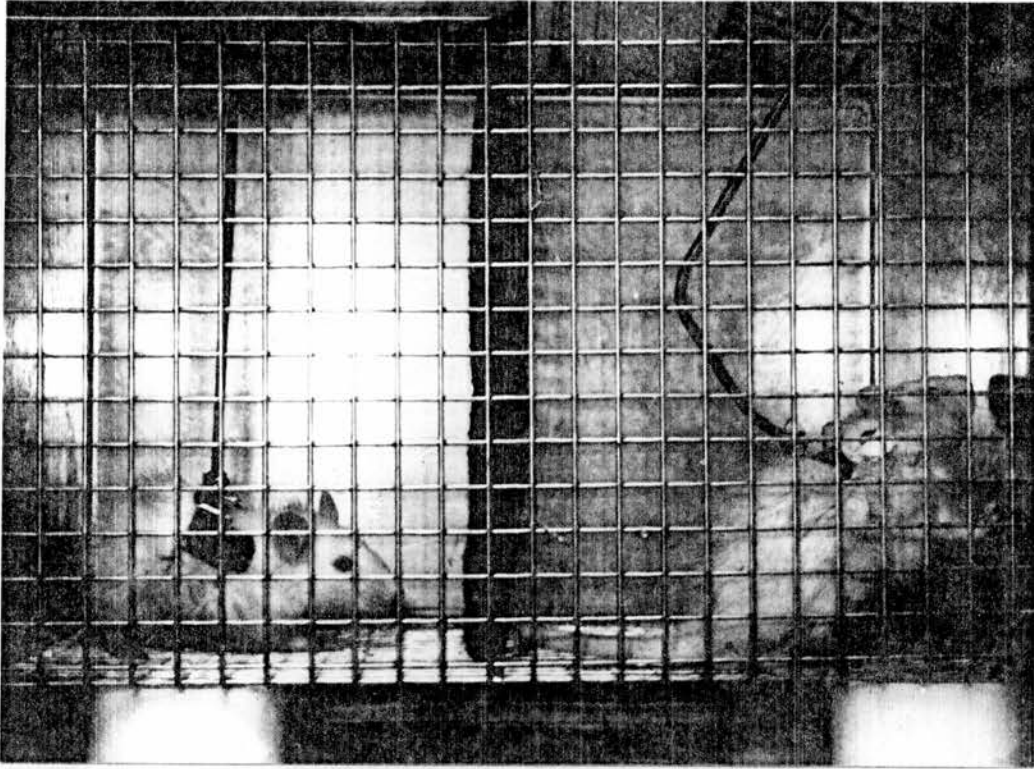


Fig. 6.1 Immature rats undergoing intravenous nutrition after a 70% intestinal resection. Note that the rats remain fully mobile during this form of feeding.

other group was allowed to drink the same intravenous solution and water *ad libitum*.

Once ten days of total parenteral nutrition, or ten days of oral feeding with the intravenous solution or rat chow had been completed, approximately half of the surviving rats were sacrificed with ten normal rats. The intravenous nutrition to the remaining 16 parenterally fed rats was reduced to 200 ml/kg/day for the next 24 hours, while they were allowed free access to the rat chow. The lines were then removed, and all of the surviving rats, no matter how they had been fed initially, were fed rat chow for the subsequent four weeks.

Intestinal specimens were removed as in Chapter Five for biochemical estimations. Five cms of the intestine was removed from 10-15 cms proximal to the anastomosis, and from 10-15 cms distal to it. For histological examination, a further one centimeter of intestine was removed from 15-16 cms proximal to the anastomosis, and from 15-16 cms distal to the anastomosis. Mucosal scrapings were taken from these older animals.

To determine if any inhibition of intestinal adaptation caused by the parenteral nutrition was temporary or permanent, all of the surviving rats were allowed free access to rat chow for the next four weeks. Then, 38 days after the intestinal resection, all of the remaining rats were sacrificed, together with the remaining eleven normal rats.

Statistical analysis was by the Student's *t* test for unpaired data.

RESULTS

Sixty-six per cent (33/50) of the animals fed by vein after 70% small-bowel resection survived, and gained weight. The remaining animals either failed to gain weight because of line disruptions or leakage, or they died of infective line complications. Seventy-six per cent (61/80) of the animals that were fed by mouth after the 70% mid-small bowel resection survived and gained weight.

SOMATIC GROWTH

In the first ten days of the study, normal rats that had not undergone surgery gained weight faster than any of the animals undergoing intestinal resection (Fig. 6.2 a & b), no matter how they were fed after the surgery ($p < .001$). Nevertheless, those fed on chow for the ten days following the surgery gained 8% more weight than those fed by vein ($p < .001$). When all of the rats were fed chow for the subsequent four weeks, however, there was

a reversal of the weight advantage for those rats fed the chow immediately after the intestinal resection. The body weight of those rats fed the i.v. solution, either by vein or by mouth, for the first ten days after intestinal resection was similar to the body weight of normal rats. But, the body weight of those rats fed the chow for the first ten days after gut resection was 15% less than the body weight of normal rats ($p < .001$), 7% less than the body weight of rats fed i.v. by vein, and 10% less than the rats fed the i.v. solution by mouth (i.v. by mouth or by vein v chow N.S.).

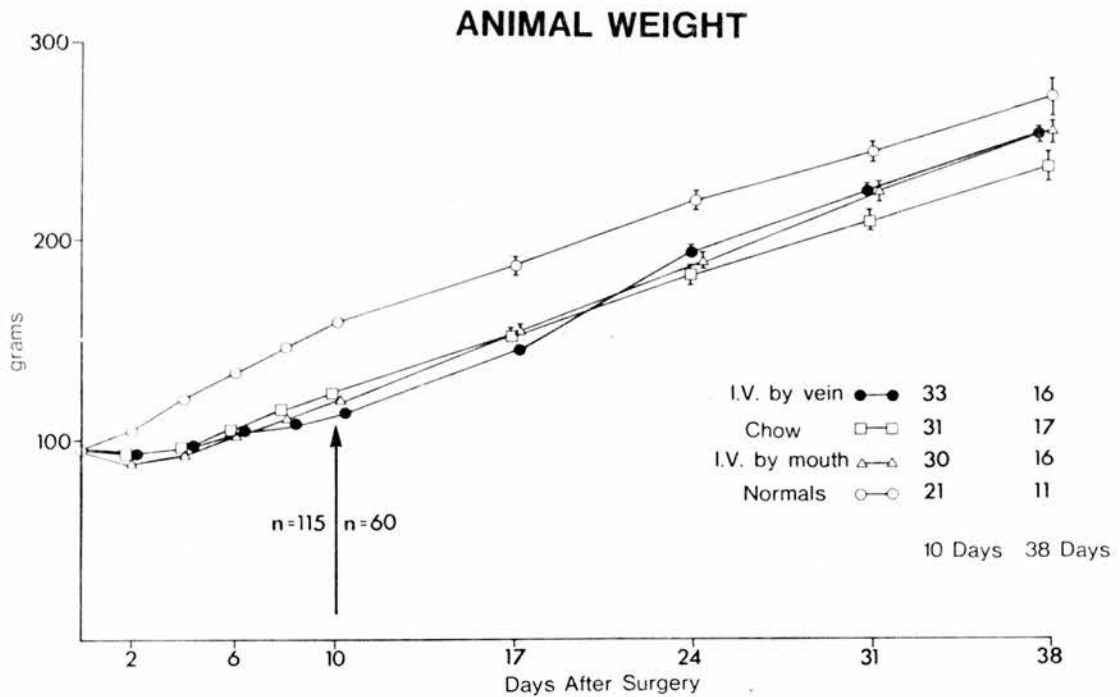


Fig. 6.2a. Animal weight (\pm S.E.). The arrow indicates the first sacrifice point when approximately half of the animals from each of the feeding regimens were sacrificed. After that, all of the remaining animals from each of the feeding regimens were fed rat chow ad libitum.

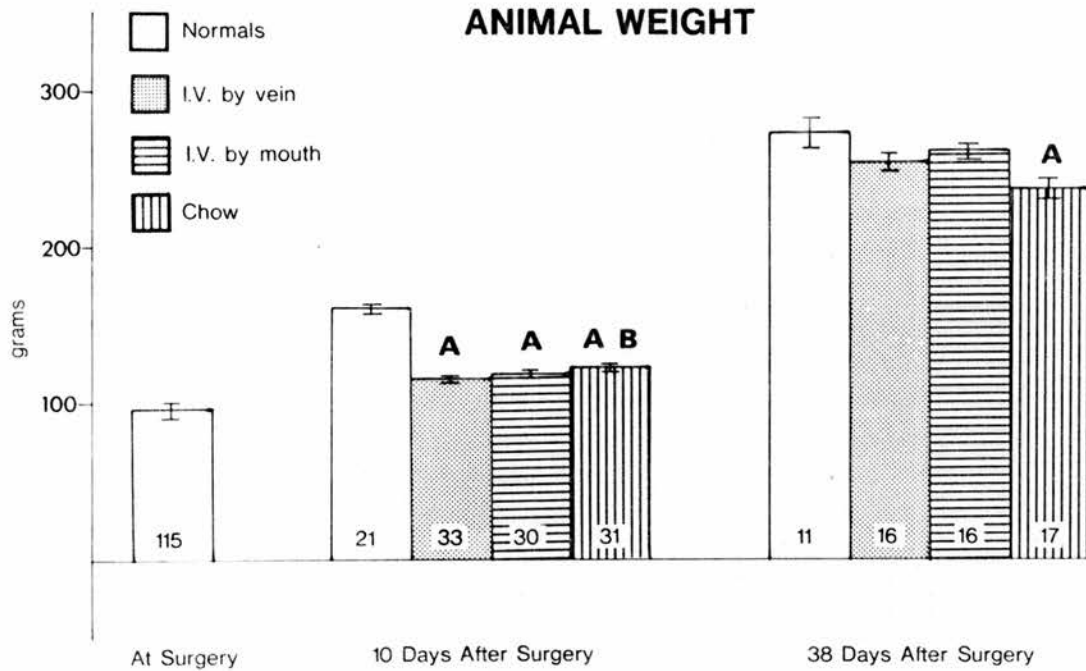


Fig. 6.2b. Animals weight (\pm S.E.) at the first and second sacrifice points. A = $p < .001$ v normal rats; B = $p < .001$ v rats fed by vein.

INTESTINAL ELONGATION

The residual intestine of those rats fed the i.v. solution, either by vein or by mouth, did not grow for the ten days after intestinal resection (Fig. 6.3), whereas the intestine in normal rats elongated by 15% (in the jejunum $p < .02$ v rats fed the i.v. by vein, and $p < .05$ v rats fed the i.v. solution by mouth. In the ileum $p < .02$ v rats fed the i.v. solution by mouth). When rats were fed chow for the first ten days after intestinal resection, the intestine elongated by 15% in the jejunum, and 29% in the ileum (in the ileum $p < .02$ v rats fed the i.v. by mouth). Despite this initial lack of intestinal elongation, when rats were fed the i.v. solution, either by vein or by mouth, for ten days, when all of the animals undergoing intestinal resection had

been allowed free access to chow for the next four weeks, compensatory elongation of the residual intestine had occurred, with 21-41% elongation over and above normal growth in the jejunum and 28-52% in the ileum.

There were, however, minor, but significant, differences in the final response to the intestinal resection that depended on the initial feeding regimen. The intestine of those rats that had been fed the i.v. solution by vein for the first ten days demonstrated catch-up longitudinal growth to such an extent over the next four weeks that the residual jejunum of those rats fed by

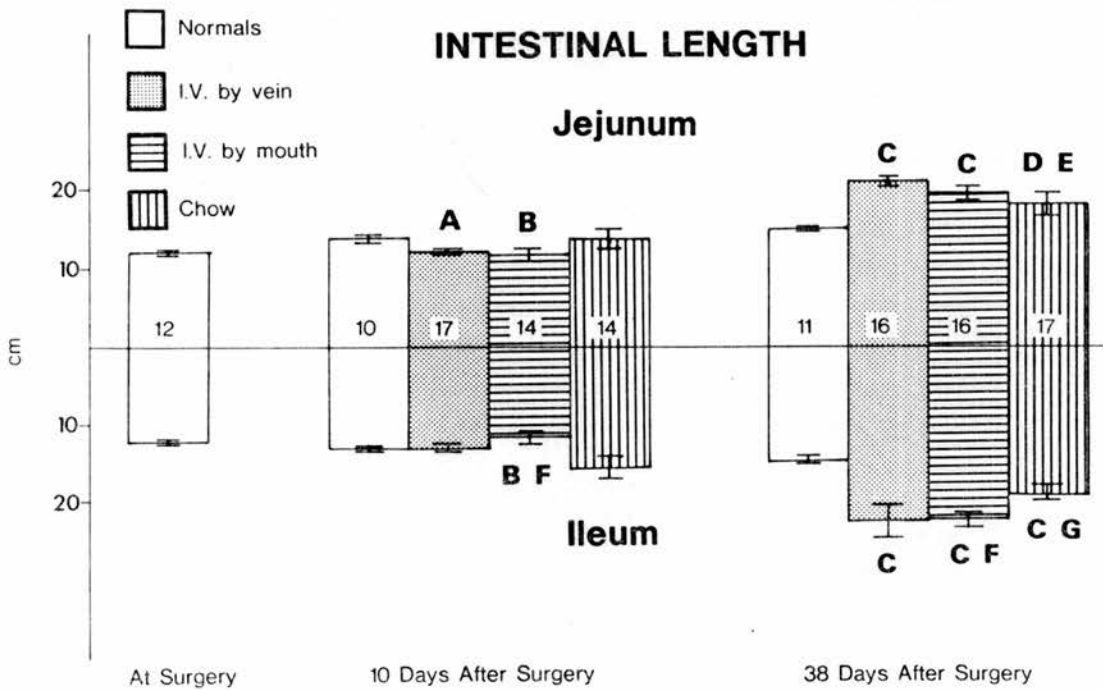


Fig. 6.3 Intestinal length (\pm S.E.) at the first and second sacrifice points. A = $p < .02$ v normal rats; B = $p < .05$ v normal rats; C = $p < .001$ v normal rats; D = $p < .01$ v normal rats; E = $p < .02$ v rats fed by vein; F = $p < .02$ v rats fed chow; G = $p < .05$ v rats fed by vein.

vein initially was no 17% longer than the residual jejunum of those fed chow initially after their intestinal resection ($p = <.02$). The residual ileum of those rats fed by vein initially was now 18% longer than in those rats fed on chow initially ($p = <.05$). After feeding rats the i.v. solution by mouth for the first ten days after intestinal resection, the final increase in the length of the ileum was 18% longer than in those fed chow initially ($p = <.02$).

MUCOSAL GROWTH

For the ten days following intestinal resection, mucosal growth was inhibited in the small-bowel of those rats fed by vein, despite the gain in body weight. When rats were fed orally, however, mucosal growth occurred even when rats were fed the i.v. solution by mouth.

VILLUS GROWTH

When rats were fed by vein, the jejunal villi failed to grow (Fig. 6.4), and were 5% shorter than normal. When rats were fed the i.v. solution by mouth, the jejunal villi were 44% taller than normal, and 52% taller than in those rats fed by vein ($p = <.001$ v normal rats and rats fed by vein). When rats were fed chow for the first ten days after surgery, the jejunal villi were 39% taller than normal ($p = <.001$), 46% taller than those rats fed by vein ($p = <.001$), and 4% taller than those fed the i.v. solution by mouth.

In the ileum, when rats were fed by vein for ten days, the villi were 13% taller than the villi of normal rats (N.S.). When rats were fed the i.v. solution by mouth, the villi were 54% taller than normal ($p = <.001$), and 37% taller than in rats fed by vein ($p = <.001$). When rats were fed chow, the villi were 57% taller than in normal rats ($p = <.001$), 2% taller than in those rats fed the i.v. solution by mouth (N.S.), and 39% taller than in those rats fed by vein ($p = <.001$).

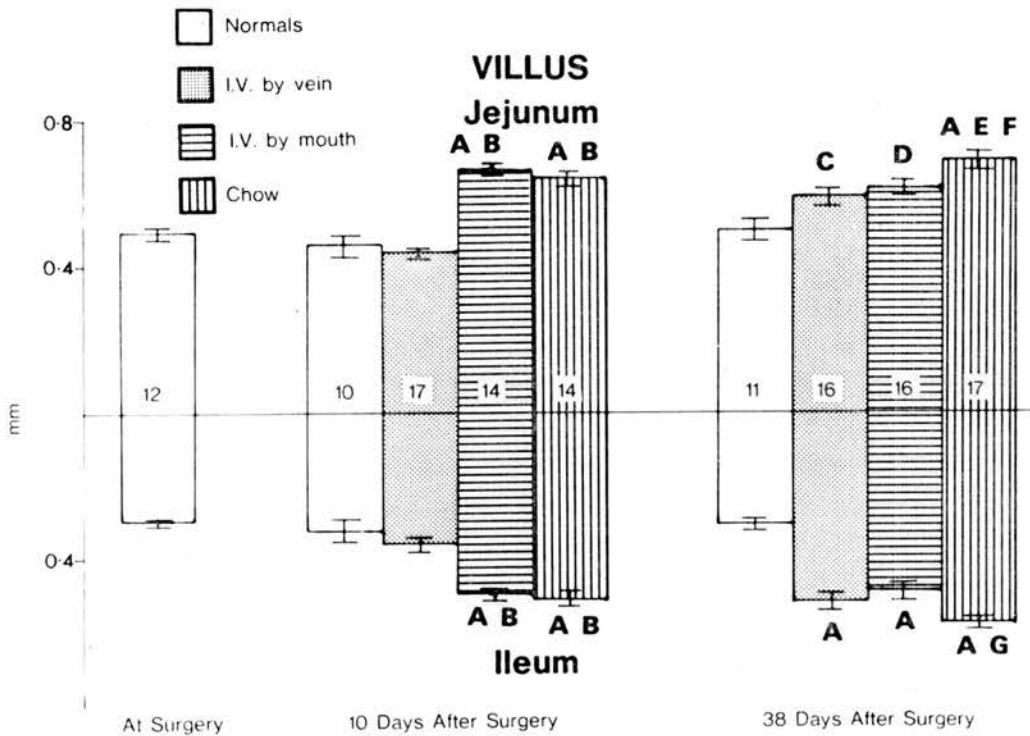


Fig. 6.4 Villus height (\pm S.E.) at the two sacrifice points. A = $\rho < .001$ v normal rats; B = $\rho < .001$ v rats fed by vein; C = $\rho < .05$ v normal rats; D = $\rho < .01$ v normal rats; E = $\rho < .02$ v rats fed by vein; F = $\rho < .05$ v rats fed i.v. solution by mouth; G = $\rho < .01$ v rats fed i.v. solution by mouth.

After the subsequent four weeks of normal feeding, villi in those rats undergoing intestinal resection demonstrated compensatory elongation, both proximal and distal to the intestinal anastomosis, no matter what feeding regimen had been followed initially, so that rats being fed by vein had almost caught up to those rats fed on chow. But, there were minor differences in the final response to the intestinal resection that depended on the initial feeding regimen.

In the jejunum of those rats initially fed by vein, the villi grew 18% more than normal ($\rho < .05$); in those rats initially fed

the i.v. solution by mouth, the villi grew 24% more than normal, and 5% more than in those rats fed by vein ($p = <.01$ v normal rats). But, in those rats initially fed chow after the intestinal resection, the jejunal villi grew 39% more than normal ($p = <.001$), 18% more than those fed by vein ($p = <.02$), and 12% more than those fed the i.v. solution by mouth ($p = <.05$).

In the ileum, in those rats that had been fed by vein initially, the villi grew 69% more than normal ($p = <.001$). In those rats fed the i.v. solution by mouth initially, the villi grew 59% more than normal ($p = <.001$), and 6% less than in those rats fed by vein (N.S.). In those rats fed chow initially after intestinal resection, the ileal villi grew 87% more than normal ($p = <.001$), 11% more than those fed by vein initially (N.S.), and 18% more than those fed the i.v. solution by mouth initially ($p = <.01$).

CRYPT DEPTH

The crypts failed to grow for the ten days following an intestinal resection when rats were fed by vein (Fig. 6.5), but they became deeper when rats were fed by mouth.

In the jejunum, the crypts were 13% shallower than normal when animals were initially fed by vein (N.S.), were 28% deeper than normal when they had been fed the i.v. solution by mouth initially ($p = <.01$ v normal, and $p = <.001$ v rats fed by vein), and were 51% deeper than normal when they had been fed chow initially for the first ten days after intestinal resection ($p = <.001$ v normal rats; $p = <.001$ v rats fed by vein; and $p = <.01$ v rats fed the i.v. solution by mouth).

Ten days after the intestinal resection, the crypts in the ileum were 31% shallower than normal when animals were fed by vein ($p = <.001$), 6% deeper than normal when rats were fed the i.v. solution by mouth ($p = <.001$ v rats fed by vein), and 46% deeper than normal when rats were fed chow ($p = <.001$ v normal rats; $p = <.001$ v rats fed by vein; and $p = <.001$ v rats fed the i.v. solution by mouth).

After the subsequent four weeks of normal feeding, the crypts in the jejunum were 66% deeper after feeding by vein initially, 64% deeper after feeding the i.v. solution by mouth, and 83% deeper after feeding chow initially ($p < .001$ for all three v normal rats). In the ileum, the crypts were 62% deeper than normal when rats had been fed by vein initially ($p < .001$), and were 29% deeper than normal when they had been fed the i.v. solution by mouth initially ($p < .01$), which, in turn, was 20% shallower than after being fed by vein initially ($p < .01$). The crypts were 62% deeper than normal when rats had been fed chow initially ($p < .001$), and 20% deeper than in those rats fed the i.v. solution by mouth initially ($p < .01$).

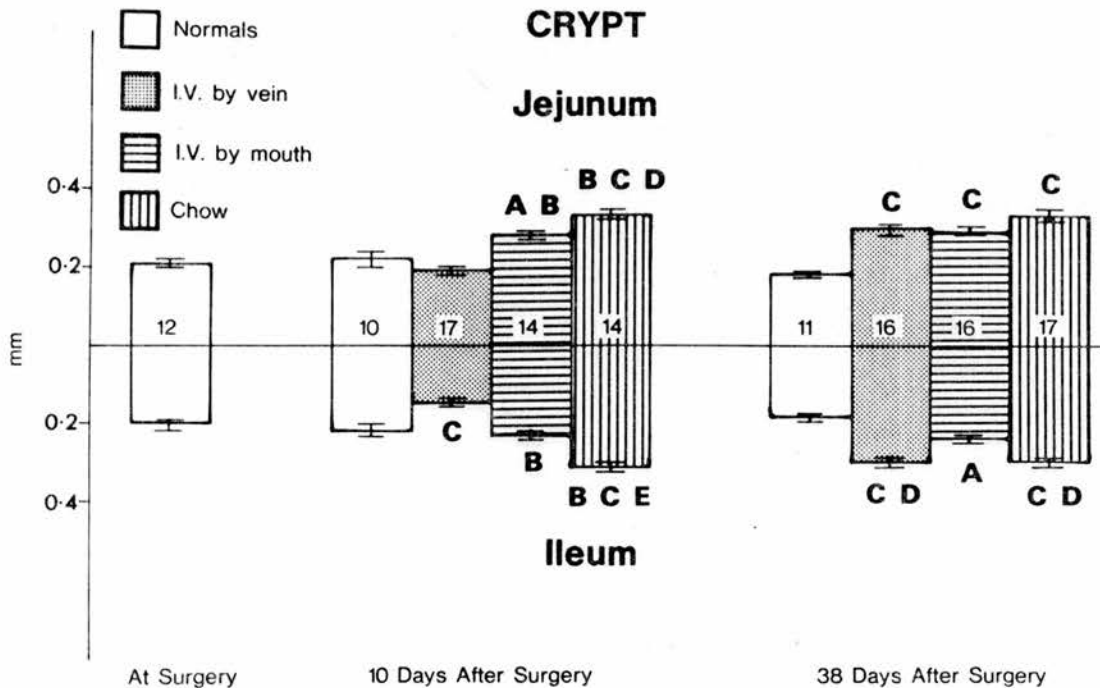


Fig. 6.5 Crypt depth (\pm S.E.) at the two sacrifice points. A = $p < .01$ v normal rats; B = $p < .001$ v rats fed by vein; C = $p < .001$ v normal rats; D = $p < .01$ v rats fed i.v. solution by mouth; E = $p < .001$ v rats fed i.v. solution by mouth.

MUCOSAL DNA CONTENT

Ten days after intestinal resection, the DNA content of the small intestine was markedly reduced when rats were fed by vein (Fig. 6.6). After feeding the i.v. solution by mouth for ten days, however, the DNA content of the intestine was essentially normal. After feeding rats chow for ten days after an intestinal resection, the DNA content of the intestine was greater than normal.

There was a 38% fall in the DNA content of the jejunal mucosa when rats were fed by vein for ten days ($p < .001$ v normal). When rats were fed the same solution by mouth, there was a 25% greater than normal DNA content ($p < .02$) and a 97% greater than normal DNA content than when rats were fed by vein ($p < .001$). When rats were fed chow for the ten days following intestinal resection, the DNA content of the jejunum was 36% greater than normal ($p < .001$), 118% greater than in rats fed by vein ($p < .001$), and 11% greater than in rats fed the i.v. solution by mouth (N.S.).

In the ileum, there was a 53% fall in the DNA content of the mucosa when rats were fed by vein for ten days ($p < .001$ v normal), and a 10% fall when rats were fed the same solution by mouth ($p < .001$ v rats fed by vein). After feeding chow for the ten days after the intestinal resection, there was an 11% increase in the DNA content in the ileal mucosa ($p < .001$ v rats fed by vein).

After the subsequent four weeks of normal feeding, there was a uniform increase in the DNA content of the mucosa after intestinal resection, with a 51-61% increase in the DNA content in the jejunum ($p < .001$ for all three v normal rats), and a 49-51% increase in the DNA content of the ileum ($p < .001$ for all three v normal rats).

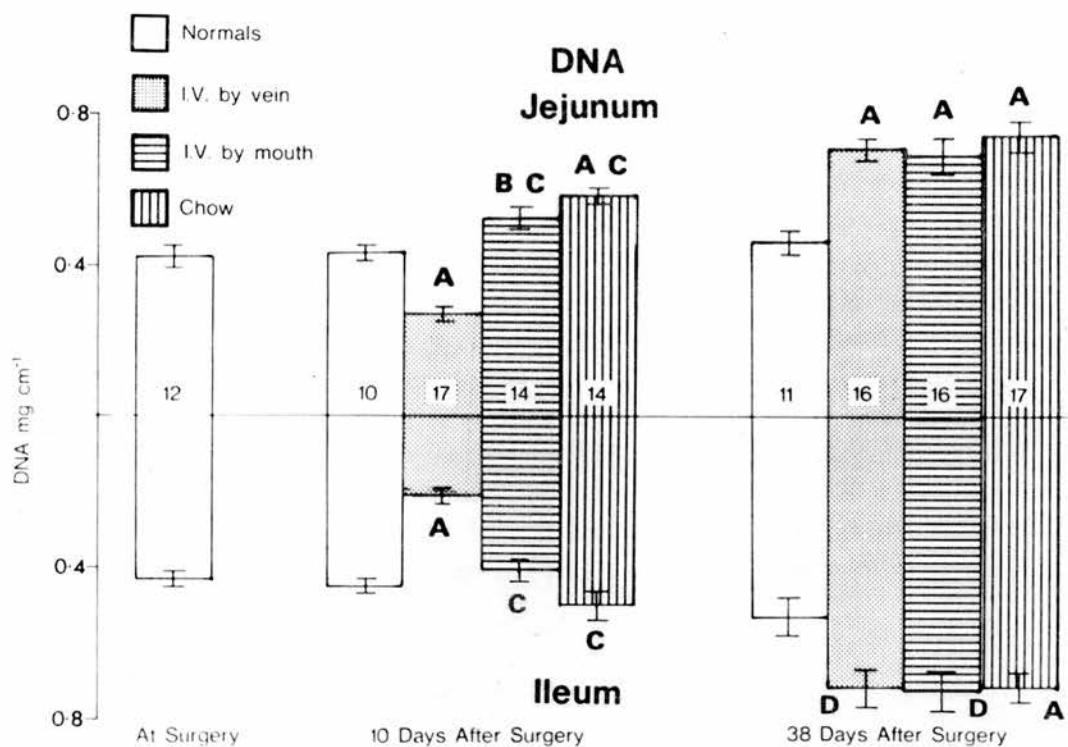


Fig. 6.6 Mucosal DNA content (\pm S.E.) at the two sacrifice points. A = $p < .001$ v normal rats; B = $p < .02$ v normal rats; C = $p < .001$ v rats fed by vein; D = $p < .01$ v normal rats.

MUCOSAL RNA CONTENT

Ten days after intestinal resection, the RNA content of the intestinal mucosa was markedly reduced when rats had been fed by vein (Fig. 6.7). When they had been fed the same solution by mouth, the RNA content was greater than normal, but when fed chow the RNA content was greater still. After the subsequent four weeks of normal feeding, however, there was an increased RNA content in the intestinal mucosa of all of the rats that had undergone intestinal resection.

Ten days after intestinal resection, the RNA content of the jejunal mucosa was 53% less than normal when animals were fed by vein ($p < .001$). When animals were fed the i.v. solution by mouth,

the RNA content of the mucosa was 81% greater than normal, and 287% greater than in those rats fed by vein ($p < .001$ v normal rats; and $p < .001$ v rats fed by vein). When rats were fed chow for the ten days following intestinal resection, the RNA content of the jejunal mucosa was 184% greater than normal, 506% greater than those fed by vein, and 57% greater than those fed the i.v. solution by mouth ($p < .001$ v rats fed by vein or the i.v. solution by mouth or normal rats).

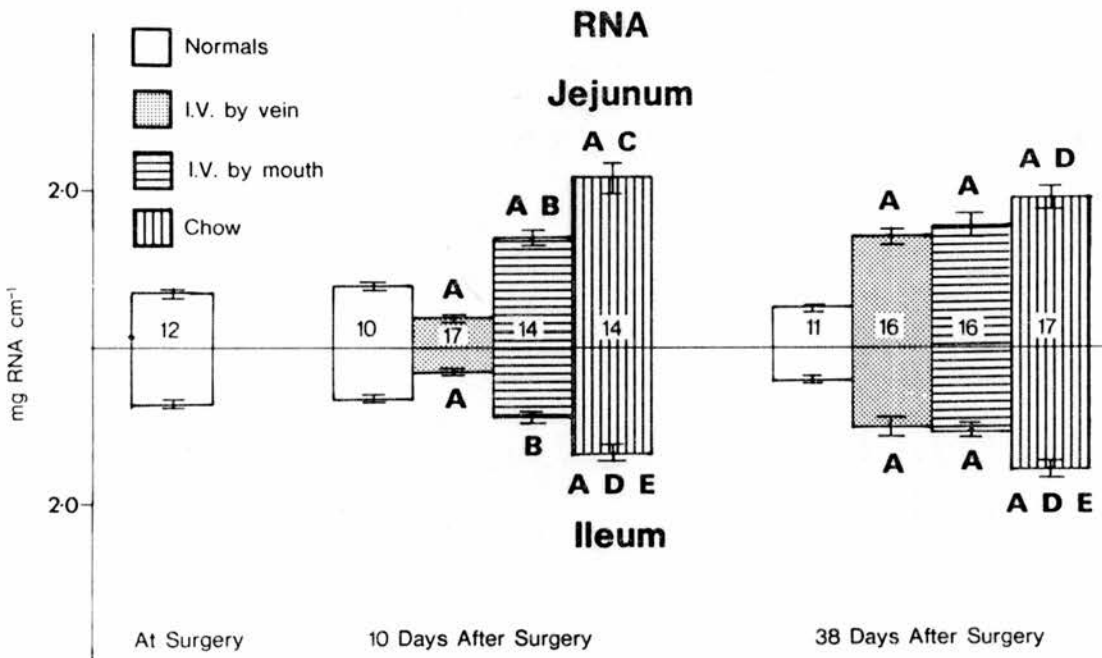


Fig. 6.7 Mucosal RNA content (\pm S.E.) at the two sacrifice points. A = $p < .001$ v normal rats; B = $p < .001$ v rats fed by vein; C = $p < .001$ v rats fed the i.v. solution by mouth; D = $p < .01$ v rats fed by vein; E = $p < .01$ v rats fed i.v. solution by mouth.

Ten days after intestinal resection, the RNA content of the ileal mucosa was 51% less than normal when animals were fed by vein ($p = <.001$), and was 23% greater than normal when rats were fed the i.v. solution by mouth (N.S.), which was also 152% greater than the RNA content of the ileal mucosa of those rats fed by vein ($p = <.001$). When rats were fed chow for the ten days following surgery, the RNA content of the mucosa was 104% greater than normal ($p = <.001$), 318% greater than in those rats fed by vein ($p = <.001$), and 65% greater than in those rats fed the i.v. solution by mouth ($p = <.01$).

After the subsequent four weeks of normal feeding, the RNA content of the intestinal mucosa of those rats initially fed the i.v. solution by vein or by mouth was generally similar, but greater than normal. The RNA content of the intestinal mucosa of those rats fed chow initially was greater still.

After four weeks of normal feeding, the RNA content of the jejunal mucosa in those rats fed initially by vein was 201% greater than in the jejunal mucosa of normal rats ($p = <.001$). In those rats initially fed the i.v. solution by mouth, the mucosal RNA content was 225% greater than normal, and 8% greater than those fed by vein ($p = <.001$ v normal). When rats had been fed chow for the initial ten days after intestinal resection, the final RNA content of the mucosa was 300% greater than normal, 23% greater than those fed the i.v. solution by mouth initially, and 33% greater than those that had been fed by vein initially ($p = <.001$ v normal and .01 v rats fed the i.v. solution by vein).

After four weeks of normal feeding, the RNA content of the ileal mucosa of those rats fed initially by vein was 153% greater than in the ileal mucosa of normal rats ($p = <.001$). In those rats fed the i.v. solution by mouth initially, the RNA content was 163% greater than normal, and 4% greater than in those fed by vein ($p = <.001$ v normal). When rats were fed chow initially, the mucosal RNA content was 287% greater than normal, 52% greater than in those fed by vein, and 47% greater than in those rats

fed the i.v. solution by mouth initially ($p = <.001$ v normal and $<.01$ v those fed the i.v. solution either by vein or by mouth).

RNA:DNA RATIOS

There were early increases in cell size after intestinal resection when cell size was expressed as RNA:DNA ratios (376, 393), but only when rats were fed by mouth (Fig. 6.8). Furthermore, some of this difference remained, for in those animals that were fed chow for the first ten days after intestinal resection the initial cell size and the final cell size were greater than in the intestinal mucosa of those rats fed the intravenous solution, by mouth or by vein, for the first ten days after the surgery.

Ten days after intestinal resection, the RNA:DNA ratio of the jejunal mucosa was 24% less than normal when rats were fed by vein ($p = <.01$). When rats were fed the same solution by mouth, the RNA:DNA ratio was 46% greater than normal, and 93% greater than in those rats fed by vein ($p = <.001$ v normal and rats fed by vein). When rats were fed chow, the RNA:DNA ratio was 107% greater than normal, 174% greater than in those fed by vein, and 42% greater than in rats fed the i.v. solution by mouth ($p = <.001$ v normal, v rats fed by vein, and v rats fed the i.v. solution by mouth).

Ten days after intestinal resection, the RNA:DNA ratio of the ileal mucosa was 23% more than normal when rats had been fed by vein (N.S.). When rats were fed the same solution by mouth, the ratio was 43% greater than normal, and 17% greater than in those rats fed by vein ($p = <.01$ v normal). When rats were fed chow, the RNA:DNA ratio was 98% greater than normal, 62% greater than in rats fed by vein, and 38% greater than in those rats fed the i.v. solution by mouth ($p = <.001$ v normals; $p = <.01$ v rats fed by vein; and $p = <.05$ v rats fed the i.v. solution by mouth).

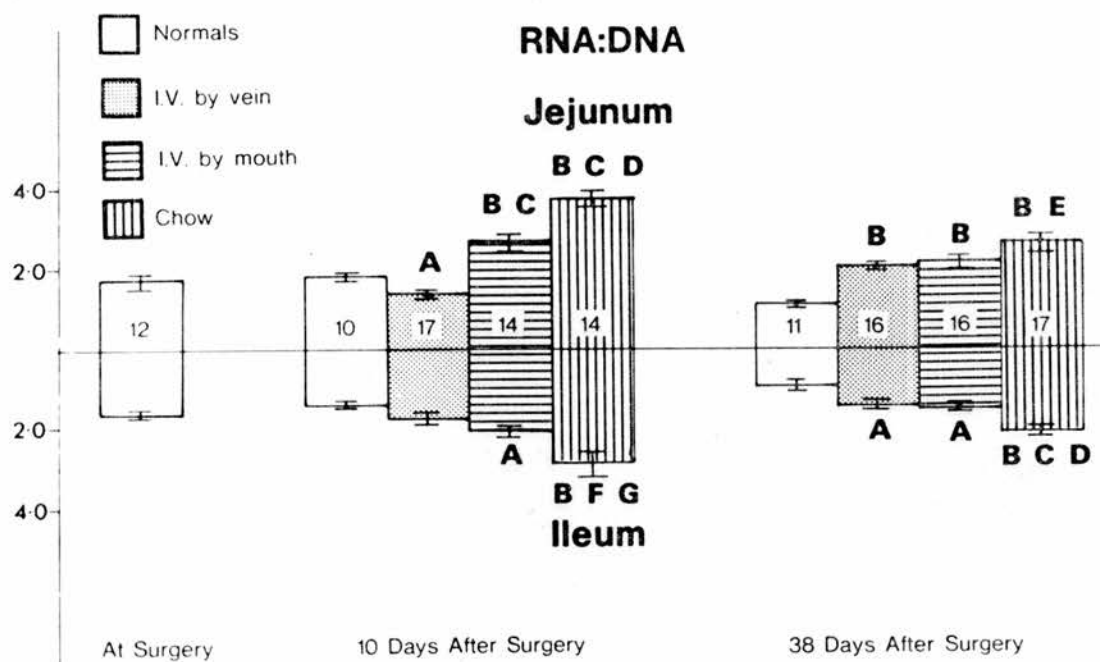


Fig. 6.8 Mucosal RNA:DNA ratios (\pm S.E.) at the two sacrifice points. A = $\rho < .01$ v normal rats; B = $\rho < .001$ v normal rats; C = $\rho < .001$ v rats fed by vein; D = $\rho < .001$ v rats fed the i.v. solution by mouth; E = $\rho < .05$ v rats fed by vein; F = $\rho < .01$ v rats fed by vein; G = $\rho < .05$ v rats fed i.v. solution by mouth.

After the subsequent four weeks of normal feeding, the RNA:DNA ratio was 91% greater than normal in the jejunal mucosa of rats fed by vein initially ($\rho = < .001$). The ratio was 98% greater than normal, and 4% greater than in rats fed by vein, when rats had been fed the same solution by mouth initially ($\rho = < .001$ v normal). When rats had been fed chow initially, the final RNA:DNA ratio was 141% greater than normal, 27% greater than in those fed by vein, and 22% greater than in those fed the i.v. solution by mouth ($\rho = < .001$ v normal; and $\rho = < .05$ v rats fed by vein).

In the ileal mucosa, the final RNA:DNA ratio was 54% greater than normal in rats fed by vein initially ($p < .01$). When rats were fed the same solution by mouth, the ratio was 61% greater than normal, and 4% greater than in those fed by vein ($p < .01$ v normal). When rats had been fed chow initially, the RNA:DNA ratio was 127% greater than normal, 47% greater than in rats fed by vein, and 41% greater than in rats fed the i.v. solution by mouth ($p < .001$ v normal and rats fed the i.v. solution either by vein or by mouth).

MUCOSAL ENZYME ACTIVITY

Sucrase activity per centimeter rose sharply, but only in the proximal small intestine ten days after an intestinal resection if rats were fed by mouth. After the subsequent four weeks of normal feeding, the increased sucrase activity per centimeter was present in all rats undergoing resection, and there was now a demonstrable response in the ileal mucosa as well (Fig. 6.9)

SUCRASE ACTIVITY PER CENTIMETER

Ten days after intestinal resection, the jejunal sucrase activity per centimeter was 38% less than normal when animals were fed by vein ($p < .01$). When animals had been fed the same solution by mouth, the activity was 112% greater than normal, and 242% greater than in rats fed by vein ($p < .001$ v normals and v rats fed by vein). When rats were fed chow, the jejunal sucrase activity was the same as those fed the i.v. solution by mouth.

Ten days after the intestinal resection, the only significant rise in sucrase activity per centimeter in the ileum was seen in those rats fed the i.v. solution by mouth ($p < .05$ v normal rats).

After the subsequent four weeks of normal nutrition, the sucrase activity per centimeter in the jejunal mucosa was 98% greater than normal in those rats that had been fed by vein in-

initially ($p < .001$). When rats had been fed the same solution by mouth, the activity was 139% greater than normal, and 21% greater than in rats fed by vein ($p < .001$ v normal). When rats were fed chow initially, the sucrase activity per centimeter was 149% greater than normal, 26% greater than in rats fed by vein, and 4% greater than in rats fed the i.v. solution by mouth ($p < .001$ v normal).

In the ileum, the mucosal sucrase activity per centimeter was 828% greater than normal in those rats that had been fed by vein initially ($p < .01$). When rats were fed the same solution by mouth initially, the activity was 353% greater than normal, and 51% less than in rats fed by vein ($p < .001$ v normal). When rats

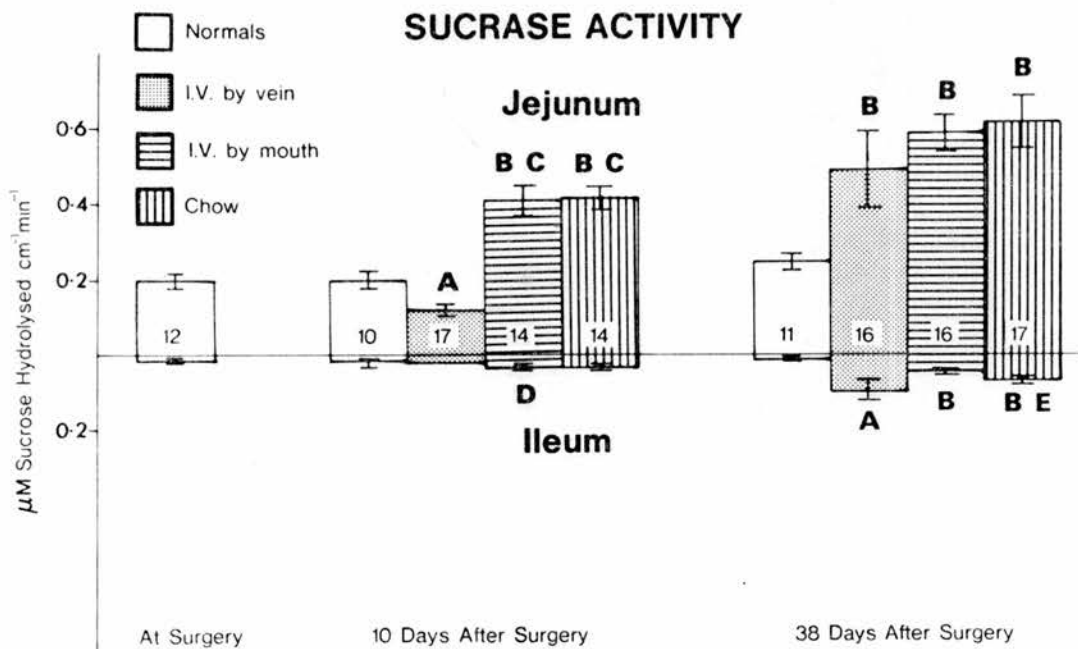


Fig. 6.9 Mucosal sucrase activity (\pm S.E.) at the two sacrifice points. A = $p < .01$ v normal rats; B = $p < .001$ v normal rats; C = $p < .001$ v rats fed by vein; D = $p < .05$ v rats fed by vein; E = $p < .05$ v rats fed i.v. solution by mouth.

were fed chow initially, the sucrase activity per centimeter was 552% greater than normal, 30% less than in rats fed by vein, and 44% greater than in rats fed the i.v. solution by mouth ($p = <.001$ v normal rats; and $p = <.05$ v rats fed the i.v. solution by mouth).

SUCRASE SPECIFIC ACTIVITY

Ten days after intestinal resection, the sucrase activity per mg DNA rose in the jejunum when rats were fed orally (Fig. 6.10). After the subsequent four weeks of normal feeding there was increased enzyme activity both proximal and distal to the anastomosis.

Ten days after intestinal resection there was no change in the sucrase activity per mg DNA in the jejunal mucosa of those rats fed by vein. When rats had been fed the same solution by mouth, the activity was 76% greater than normal ($p = <.001$ v normal rats and rats fed by vein). When rats were fed chow, the sucrase activity per mg DNA was 50% greater than normal, 15% less than those fed the i.v. solution by mouth, and 54% greater than in those fed by vein ($p = <.01$ v normal; and $p = <.001$ v rats fed by vein).

In the ileum, there were no significant increases in the sucrase activity per mg DNA within the ten days following intestinal resection.

After the subsequent four weeks of normal feeding, the jejunal sucrase activity per mg DNA was 24% greater than normal when rats had been fed by vein initially ($p = <.02$). When rats had been fed the same i.v. solution by mouth initially, the activity was 53% greater than normal, and 23% greater than in those rats fed by vein ($p = <.001$ v normal). When rats were fed chow initially, the sucrase activity per mg DNA was 50% greater than normal, 21% greater than in rats fed by vein, and 2% greater than in rats fed the i.v. solution by vein ($p = <.05$ v normal).

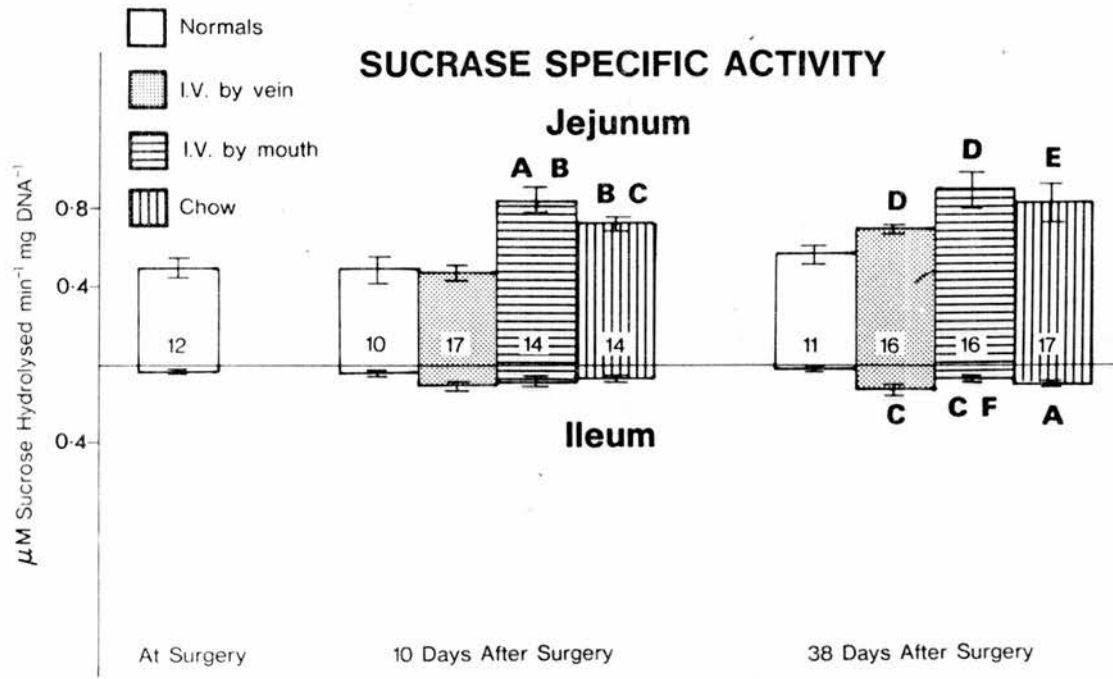


Fig. 6.10 Sucrase activity per mg DNA (\pm S.E.) at the two sacrifice points. A = $p < .001$ v normal rats; B = $p < .001$ v rats fed by vein; C = $p < .01$ v normal rats; D = $p < .02$ v normal rats; E = $p < .05$ v normal rats; F = $p < .05$ v rats fed by vein.

In the ileum, the sucrase activity per mg DNA was 526% greater than normal in those rats fed by vein initially ($p < .01$). When rats were fed the same solution by mouth initially, the final activity was 205% greater than normal, and 99% less than in those rats fed by vein initially ($p < .01$ v normal; and $p < .05$ v rats fed by vein). When rats had been fed chow initially, the final sucrase activity per mg DNA in the ileal mucosa was 347% greater than normal, 26% less than in rats fed by vein, and 46% greater than in rats fed the i.v. solution by mouth ($p < .001$ v normal).

DISCUSSION

This study demonstrates that even a short period of parenteral nutrition inhibits intestinal adaptation and longitudinal growth. The inhibition was, however, only temporary, and disappeared after the subsequent four weeks of normal feeding. This implies that a short period of feeding exclusively by vein is not detrimental to the long term response to massive intestinal resection carried out during a rapid growth period.

Nevertheless, food is vital to the normal growth and functional development of the intestinal tract. When neonatal animals suckle, the intestine grows rapidly, and the mucosal mass increases (131, 378). Furthermore, both of the bypass studies showed that, when food is excluded from the intestinal lumen of suckling rats, normal longitudinal and mucosal growth does not occur, even when normal growth occurs in the remainder of the intestine, and there is normal somatic growth. The bypass studies also showed that the normal physiological and functional development of the intestinal mucosa is inhibited in the bypassed ileal segments. Moreover, when older, but still immature, rats are fed by vein for ten days, normal growth of the intestinal mucosa does not occur (258), and when puppies are fed by vein, inhibition of normal longitudinal growth of the intestine also occurs, as well as the inhibition of mucosal growth (194).

Not only is food vital to normal intestinal growth and intestinal development, but food is also vital for maximum compensatory regeneration that occurs in the residual small bowel after intestinal resection in both mature and immature animals (101, 250, 258, 391). For example, when immature rats are fed by vein for ten days after intestinal resection, compensatory mucosal growth within the residual intestine is inhibited (258). Because of this observation, Morin *et al* speculated that early luminal nutrition was essential for the induction of a maximal response to intestinal resection in immature rats. But, the possibility of catch-up growth occurring after the animals resumed normal feeding was not considered (258). This study has demonstrated

that, in growing rats, catch-up in total body growth, and catch-up in compensatory intestinal growth occurs after an initial ten day period of growth inhibition. Even so, this study has, nevertheless, demonstrated that minor differences in the final response to intestinal resection remained, and that these differences depended on the initial feeding regimen employed immediately after the partial enterectomy. Body growth and longitudinal intestinal growth were maximal after initially feeding the i.v. solution by vein or by mouth for ten days, followed by four weeks of normal nutrition. Mucosal growth, on the other hand, especially growth of cell size, was maximal when rats were fed chow for the whole of the post-operative period. These results support previous observations that both long-term somatic growth and long-term intestinal growth are improved by early supplementary intravenous feeding after massive intestinal resection in growing animals (391). In this present study, however, an orally administered elemental diet was as effective in supporting both somatic growth and, in the long term, intestinal adaptation. Furthermore, judging from the less marked distal intestinal hyperplasia seen initially in the rats fed on the elemental diet, that diet appeared to be more effectively absorbed before it could get to the distal intestine and exert its growth promoting effects.

The increase in the mucosal DNA content, and the increasing RNA:DNA ratios, demonstrated that the mucosal response to intestinal resection included both cellular hypertrophy and cellular hyperplasia in these older animals, but only as long as food was present within the intestinal lumen. Again, there were minor differences between those animals fed chow after intestinal resection and those fed the i.v. solution. Whatever the final differences between groups, however, these were small in comparison with the differences between those rats undergoing intestinal resection and normal control rats fed rat chow.

The changes in the DNA content support previous observations that luminal nutrition is essential to produce maximal cellular hyperplasia in response to an intestinal resection (85, 101, 250,

258). Changes in the RNA:DNA content do not, however, correspond to the changes observed after intestinal resection in more mature rats, where a decrease in enterocyte cell size has been recorded (376). After intestinal resection at 10 days of age, however, a similar increase in the RNA:DNA ratio occurs (see Chapter Ten), suggesting that the enterocytes of the suckling and the immature rat are capable of cellular hypertrophy, as well as hyperplasia, in response to an intestinal resection, whereas the enterocytes of the more mature rats can only respond with cellular hyperplasia.

The reason for the final differences in the mucosal response to intestinal resection and the various feeding regimens are unclear, but the lack of longitudinal growth observed in the rats fed chow for the ten days following surgery may well have acted as an added stimulus to mucosal growth. Therefore, those rats fed the i.v. solution by mouth or by vein initially have more residual small intestine than those fed chow initially, who, in turn, have more cells per centimeter of intestine to compensate for this lack of longitudinal growth.

Enzyme activity within the residual intestine also depended on the initial nutritional regimen. Ten days after intestinal resection, sucrase activity per centimeter rose dramatically in the proximal intestine, but only when these rats were being fed by mouth. This response to intestinal resection in the immature rat demonstrated that, as in the mature rat, luminal nutrition is an important stimulus to the compensatory increase in intestinal mucosal enzyme activity, as well as compensatory mucosal growth (42, 56, 85, 149, 258, 381).

The absence of carbohydrate from the intestinal lumen for the ten days following small-bowel resection produced a marked reduction in the sucrase activity per centimeter in the jejunum, but no such reduction in sucrase activity per centimeter occurred in the ileum. This lack of reduction in the sucrase activity of the ileum supports previous observation that there is little undigested carbohydrate present in the distal small-bowel in this older rat and,

as a result, the distal sucrase activity is normally low (85, 359). As a result, when luminal carbohydrate is absent, no decrease in disaccharidase activity would be expected.

Four weeks of normal feeding changed the pattern of the enzyme activity within the residual intestine from that observed at the end of the initial ten day period. After four weeks, the sucrase activity per centimeter was the same in all of the rats that had undergone intestinal resection, no matter what the initial feeding regimen had been. In the distal small-bowel, although there was no significant response in the first ten days after resection, after the subsequent four weeks of normal feeding, there were significant increases in sucrase activity in all of the rats that had undergone intestinal resection. Although the absolute values for sucrase activity in the distal intestine never caught up to those in the proximal intestine, the increase in the sucrase activity per centimeter distally represented an eight-fold increase over normal values, whereas the increase in the proximal intestine was only a two-fold increase over normal values. This greater response distally is the result of greater concentrations of luminal nutrition reaching the distal small intestine when proximal intestine is removed (85), as the distal intestine does not normally receive large quantities of partly digested nutrients and pancreatico-biliary secretions (12, 384). The jejunum, on the other hand, does not respond so dramatically to intestinal resection, as it is normally stimulated by a high concentration of these nutrients and pancreatico-biliary secretions (85, 381).

Despite the lack of sucrase in the i.v. solution, there was no difference in the intestinal sucrase activity between rats fed chow, a diet containing sucrose, and those rats fed the i.v. solution by mouth, a diet containing glucose as its only carbohydrate source. This non-specific response in the intestinal sucrase activity has also been recorded in weanling rats fed on a diet containing a high concentration of lactose (149), and appears to be a non-specific response to a high carbohydrate load within the intestinal lumen.

Sucrase activity per mg DNA indirectly reflects the enzyme activity per cell, as the DNA content per cell remains constant (362), even at a time when the cell size is changing rapidly in response to intestinal resection (85, 376). Enzyme activity per mg DNA is, therefore, a more accurate indicator of activity per cell than activity expressed per mg protein. The changes in the enzyme activity per cell were similar to the changes in the enzyme activity per centimeter, with one exception. The sucrase activity per mg DNA did not drop when animals were fed by vein. This lack of decreased activity per cell demonstrates that the decrease in the enzyme activity per centimeter seen after i.v. feeding was simply the result of a decrease in the cell numbers, and not a decrease in sucrase activity in the individual cell. Furthermore, the increase in the sucrase activity per cell recorded here after intestinal resection does not correspond to the decrease in sucrase specific activity in the intestinal mucosa in more mature animals (376). Therefore, the increases recorded here possibly reflect the increased cell size which, again, is not observed after partial enterectomy in older animals (381).

Because of the catch-up growth in body weight, intestinal length, villus height, DNA and RNA content, and the catch-up in the mucosal sucrase activity in the residual intestine, a short period of inhibition of intestinal regeneration does not appear to be detrimental to the long-term response to intestinal resection in immature growing rats. Therefore, it is possible to speculate that a corresponding period of intestinal growth inhibition in the human neonate would not be detrimental to the long-term response to massive intestinal resection.

SECTION THREE

GROWTH FACTORS

CHAPTER SEVEN

HIGH AND LOW SERUM GASTRIN

In an attempt to improve the intestinal adaptation that follows intestinal resection in young growing animals, the effects of two gastrointestinal polypeptides that have been shown to stimulate intestinal cellular proliferation were studied: the first, gastrin, and the second, epidermal growth factor. Furthermore, one further poorly identified growth factor has been identified in breast milk, and the effects of breast milk feeding after intestinal resection were also studied (see Chapter Nine).

Exogenous and endogenous gastrin appears to be trophic to the gastrointestinal tract under two sets of circumstances. Gastrin appears to maintain mucosal mass (177), and is also implicated as an aetiological factor in the mucosal maturation that takes place as rats wean (222).

There is abundant evidence that pentagastrin maintains mucosal mass. Pentagastrin injections promote DNA synthesis and increase DNA content within the oxyntic glands and the duodenal and colonic mucosa when injected into fasted animals (170), and those undergoing antrectomy (78). Both high levels of endogenous serum gastrin and pentagastrin injections increase DNA synthesis and DNA content in chemically induced cancers (248). Luminal pentagastrin also promotes DNA synthesis, and an increased DNA and RNA content, when infused into the ileum of rats (178). Furthermore, intravenously infused pentagastrin prevents the gastric and small-intestinal atrophy associated with parenteral nutrition (177).

The evidence for an aetiological role in the process of mucosal maturation is less convincing. Nevertheless, the suckling rat has low levels of antral gastrin, which at 15-18 days of age abruptly increase to adult values (222, 402), with a synchronous onset of intestinal mucosal maturation (147, 255, 339). Therefore, gastrin has been implicated in the increases in intestinal weight, intestinal RNA, and intestinal protein content that occur as the intestinal mucosa matures (222), especially as other hormones have been shown to induce premature mucosal maturation (147, 150, 180, 181, 199, 200, 255). A further suggested role for gastrin is that of an hormonal mediator of the intestinal response to intestinal resection (172).

In the first of three experiments on intestinal polypeptides, the serum levels of endogenous gastrin were altered by antrectomy, or fundectomy, to determine if high or low levels of endogenous serum gastrin would influence intestinal growth in ten day old suckling rats, and also if the same alterations in endogenous gastrin levels would influence the normal ontogenic development of the intestinal mucosa. To do this, we reproduced the *in vivo* animal model whereby the endogenous levels of serum gastrin are altered by surgical manipulations of the stomach (274). After an antrectomy that includes the duodenal bulb (130), serum gastrin levels fall, as the majority of the gastrin producing cells are removed. Nevertheless, gastrin producing cells can still be found in the duodenum of the suckling rat (208). After fundectomy, serum gastrin levels increase, as acid production falls, and gastrin secretion continues.

MATERIALS AND METHODS

For this experiment, 10 day old rats were again used, and prepared for surgery as in the previous studies. Partial gastric resections were carried out as outlined above, and as demonstrated in Fig. 7.1. As a control, both for the loss of the reservoir capacity of the stomach and for the stress of the surgery, rumenectomy (removal of the reservoir portion of the stomach; see Fig.

7.1) was performed on a further group of 10 day old rats. Rats were then sacrificed at 15 days of age, the beginning of the weaning period (147); at 21 days of age, the middle of the weaning period; and at 27 days of age, the end of the weaning period. So as to provide a chronological profile of the changes that took place during the subsequent mucosal maturation, normal 10, 15, 21, and 27 day old rats were also studied.

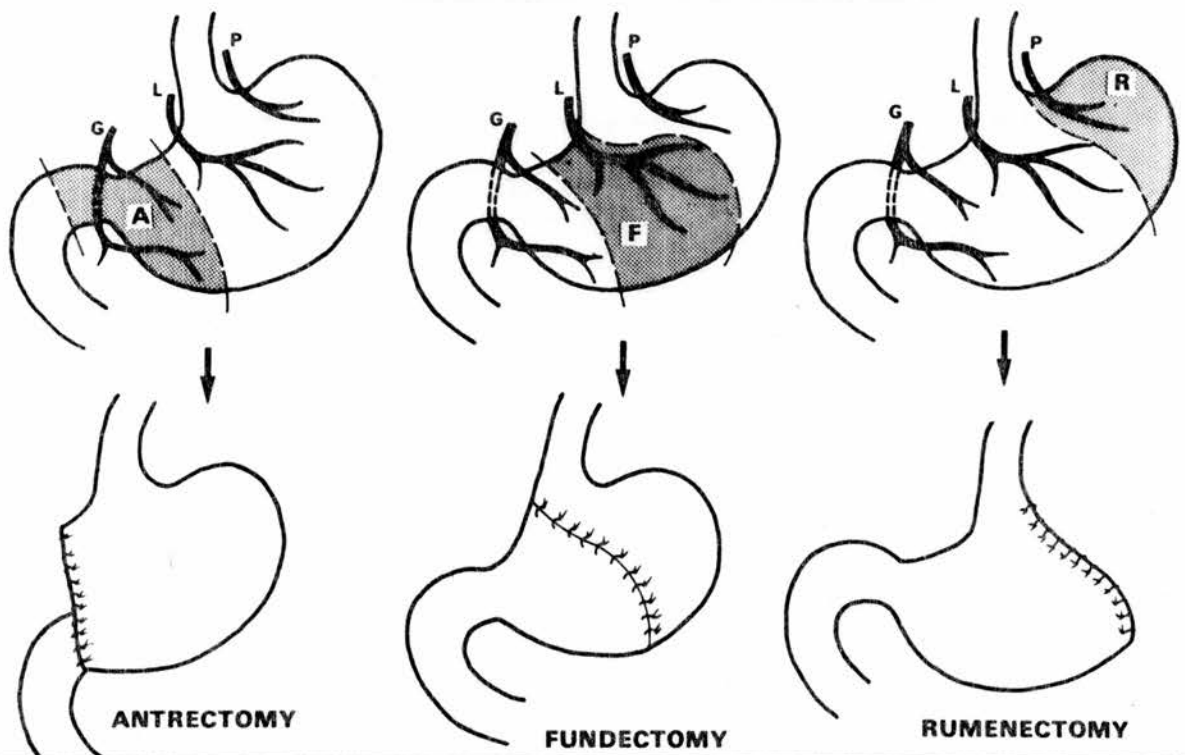


Fig. 7.1 The partial gastrectomies. A = antrectomy which entails removal of the antrum and the duodenal bulb based on the blood supply of the gastroduodenal artery (G); F = fundectomy which entails removal of the fundus based on the blood supply of the left gastric artery (L) with preservation of the oesophago-gastric junction; R = rumpectomy which entails removal of the rumen based on the blood supplied by a branch of the phrenic artery (P). Note that the final volume of the stomach was approximately equal after the three forms of partial gastrectomy.

Under ether anaesthesia, caval blood was withdrawn until rats were exsanguinated. This blood was immediately cooled on ice, centrifuged, and the serum frozen for later assay of serum gastrin levels. Duplicate radio-immunoassay was performed on coded samples of serum, using rabbit antibody to synthetic human gastrin as the probe (290; 2-7 SHG; ICI, Wilmslow, England).

Immediately after exsanguination, the entire small intestine and colon were removed, rinsed in ice cold saline solution, and measured suspended with a 5 g weight at the distal end.

Histological specimens were taken from 5-6 cm distal to the ligament of Treitz (jejunum), and from 1-2 cm distal to the caecum (colon). Mucosal scrapings were obtained from 6-11 cm distal to the ligament of Treitz, and from 2-7 cm distal to the caecum. Pups that had gained weight poorly, or failed to demonstrate the appropriate increase or decrease in endogenous gastrin levels, were excluded from the study. As a result, approximately 14% of the original animals, evenly distributed between the three experimental groups, were excluded.

DNA content of the mucosal scrapings was assayed by the method of Burton (58). Sucrase activity was assayed using a gloostat reagent (352, 353). For morphological assessment, the mean value of the height of five complete villi, and the mean depth of five complete crypts, were recorded for each specimen, after longitudinal histological sections had been stained with H & E.

Statistical significance was assessed by the Student's *t* test for all but the sucrase specific activity in the 15 day old rats. In this group, the Wilcoxon rank test was used to demonstrate whether or not early or late appearance of sucrase activity was significant. All results were also analysed by a two-way analysis of variance adjusted for the gastrin level as a covariant. This analysis confirmed the results of the student test.

RESULTS

After gastric surgery, rats weighed less than their unoperated contemporaries at 21 and 27 days of age, but not at 15 days of age (Table 7.1). Nevertheless, after rumenectomy, 27 day old rats were heavier than rats that had undergone antrectomy or fundectomy. As expected, antrectomy decreased serum gastrin levels in comparison with those of normal rats and those that had undergone rumenectomy (Fig. 7.2), and these levels were consistently low for the rest of the study period. Fundectomy, on the other hand, raised the serum gastrin levels, which then continued to increase with age. In normal rats, however, there was no evidence of an abrupt increase in the serum gastrin levels at the time of weaning. After rumenectomy, serum gastrin levels remained normal.

Table 7.1 Weight at sacrifice (mean \pm S.E.)

	15 Days (g)	21 Days (g)	27 Days (g)
Control	27.2 \pm 2.6	51.0 \pm 1.3 ^a	65.4 \pm 3.7 ^b
Rumenectomy	27.4 \pm 0.5	40.1 \pm 1.4	61.5 \pm 1.1 ^c
Fundectomy	26.6 \pm 1.1	41.2 \pm 1.5	55.1 \pm 1.3
Antrectomy	27.4 \pm 1.2	37.8 \pm 2.1	54.5 \pm 3.5

a $p < 0.001$ versus operated groups

b $p < 0.02$ versus fundectomy, $p < 0.05$ versus antrectomy

c $p < 0.005$ versus fundectomy

Gut length in the rats undergoing surgery was significantly shorter than intestinal length in normal rats (Fig. 7.3), and this difference increased with age. Alterations of the serum gastrin levels were not associated with the alterations in the intestinal length, as the gut length was similar in all three groups of rats undergoing partial gastrectomy.

Villus height and jejunal crypt depth increased with age in all groups (Fig. 7.4). At 15 days of age there were small increases in jejunal crypt depth after surgery, but these had disappeared by 21 days of age. In normal 27 day old rats, however,

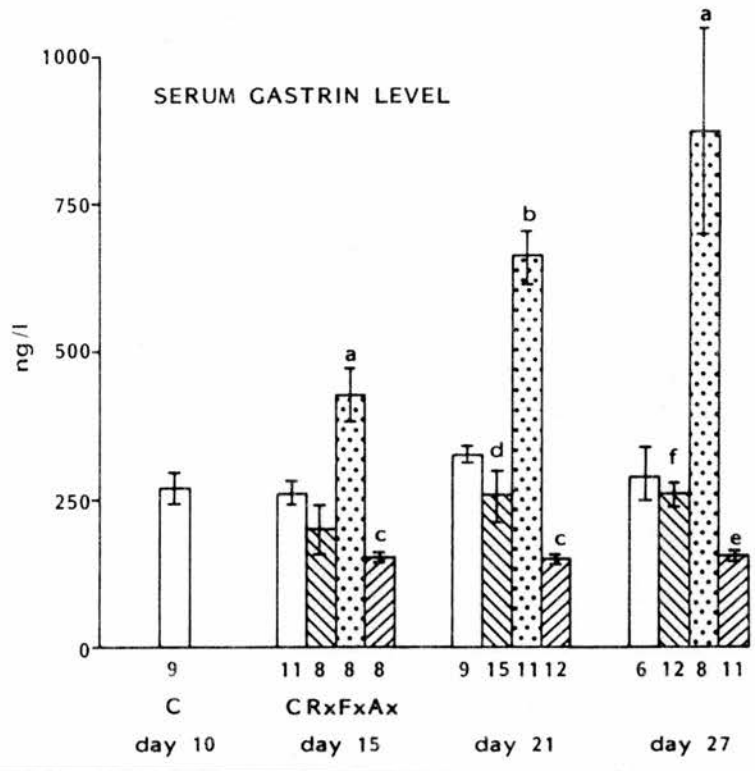


Fig. 7.2 Serum gastrin levels (\pm S.E.). A = $p < .01$ v all other groups at day 15; B = $p < .001$ v controls; C = $p < .01$ v antrectomy; D = $p < .001$ v all other groups at days 21 and 27; E = $p < .001$ v antrectomy; F = $p < .02$ v controls.

the jejunal crypt was deeper than that in any of the rats that had undergone gastric resections, but possibly, as a reflection of the increased body weight, the jejunal crypt in those rats that had undergone rumenectomy was also deeper than in those that had undergone antrectomy or fundectomy. In the colon, the response to surgery was extremely variable, with an early increase in crypt depth in those rats that had undergone rumenectomy and, to a lesser extent, those that had undergone fundectomy. This response was not maintained, so that by 27 days of age there was no difference between any of the groups studied.

All gastric resections produced a fall in the mucosal DNA content of the jejunum in comparison with normal rats of the same age, and this difference increased with age (Fig. 7.5). Those that had undergone fundectomy, and therefore those with the highest serum gastrin levels, had the lowest jejunal DNA content at 15 and 27 days of age. In the colon, the results were again variable, but all of the gastric resections apparently produced a gradual increase in mucosal DNA content, no matter which partial gastrectomy had been performed.

The sucrase specific activity increased with age, and the mean value tripled between the fifteenth and the twenty-seventh day of life (Fig. 7.6). Fundectomy, however, delayed the normal abrupt increase in sucrase activity that occurs between the sixteenth and the twentieth day of life (147, 233).

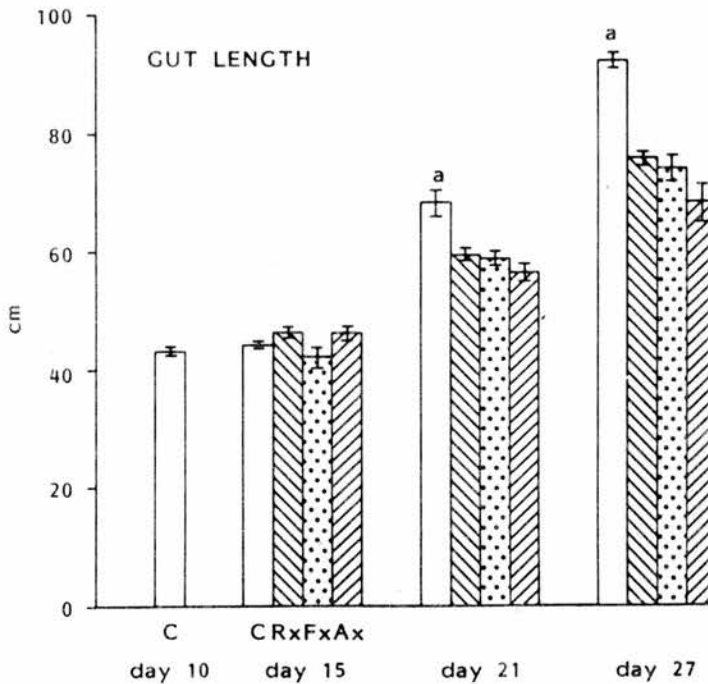


Fig. 7.3 Gut length (\pm S.E.). A = $p < .001$ v all other groups at the same age.

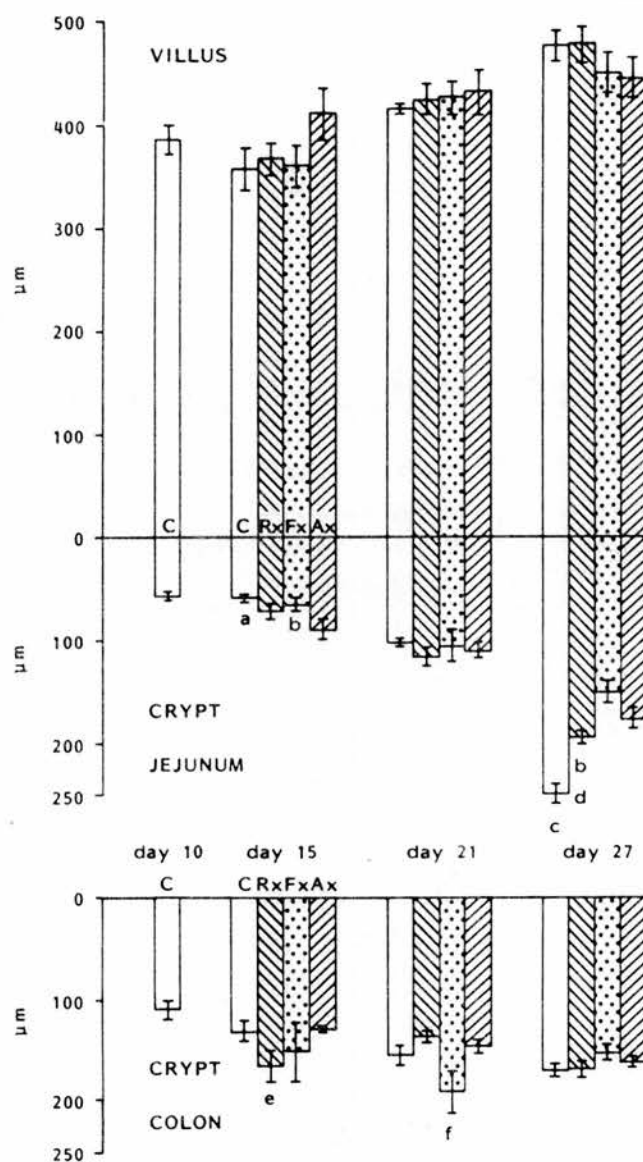


Fig. 7.4 Villus height and crypt depth in the jejunum and colon (\pm S.E.). A = $p < .001$ v antrectomy; B = $p < .02$ v antrectomy; C = p v all other groups at that age; D = $p < .005$ v fundectomy; E = $p < .05$ v antrectomy; F = $p < .001$ v rumenectomy and antrectomy, and also F = $p < .01$ v controls.

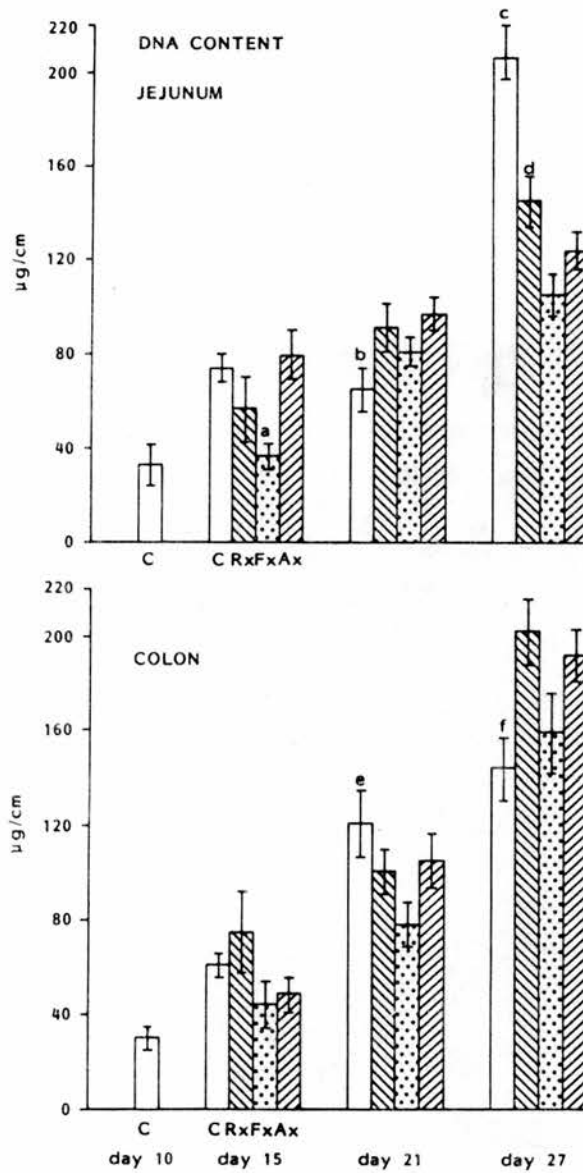


Fig. 7.5 DNA content (\pm S.E.). A = $p < .005$ v control and antrectomy; B = $p < .02$ v antrectomy; C = $p < .01$ v all other groups at the same age; D = $p < .05$ v fundectomy; E = $p < .05$ v fundectomy; F = $p < .05$ v rumenectomy and antrectomy.

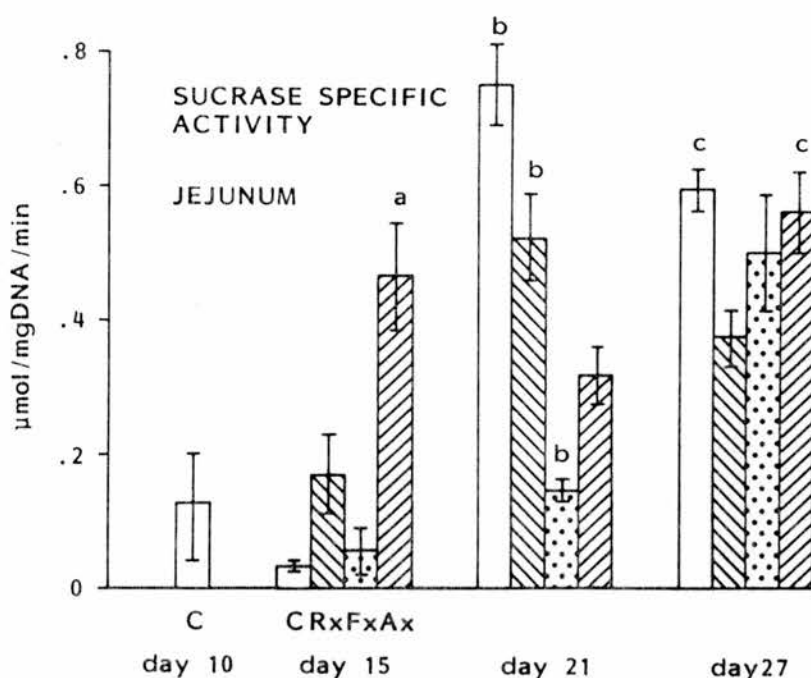


Fig. 7.6 Sucrase specific activity (\pm S.E.). A = $p < .02$ v all other groups at that age; B = $p < .02$ v all other groups at the same age; C = $p < .05$ v rumenectomy. For the results at day 15, the Wilcoxon rank sum test was used; for the other time points, Student's *t* test was used.

DISCUSSION

Despite a greater than four-fold increase in the circulating levels of gastrin, neither increased intestinal mucosal growth nor accelerated mucosal maturation could be demonstrated. Furthermore, the abrupt increases in antral gastrin levels recorded at weaning in normal rats (222) are not reflected in the serum levels during the same period. To the contrary, in those rats with the highest serum gastrin levels we demonstrated that there was an initial inhibition of the normal rise in jejunal sucrase activity that occurs at weaning, and furthermore, there was also a decreased DNA content in the jejunal mucosa of those rats. Therefore, these results are more in keeping with the increased duodenal DNA syn-

thesis observed in newborns after exposure to the effects of low maternal gastrin levels *in utero* (221).

This study demonstrates that endogenous gastrin levels can be altered in the suckling, as well as the mature rat. Nevertheless, possibly as a result of a large population of gastrin secreting cells in the duodenum and the pancreas (208), the suckling rat maintains a higher serum gastrin level after antrectomy than the older rat.

Neither body weight nor intestinal length were influenced by high or low serum gastrin levels. No matter whether antrectomy or fundectomy was performed, rats failed to gain weight normally for the 17 days following surgery, although by 27 days of age those rats undergoing fundectomy regained a normal body weight. Apart from the lack of somatic growth, intestinal elongation was also inhibited by all three gastric resections and, despite the catch-up in body weight after fundectomy, there was no similar catch-up in intestinal length.

Alterations in the serum gastrin levels failed to influence villus growth, as no difference in jejunal villus height was apparent between any of the rats at any stage, suggesting that jejunal villus height, at least, is independent of serum gastrin levels, body weight, or intestinal length. These results confirm observations that, where there is no loss of absorptive surface area, jejunal villus height appears to be controlled by local factors (9, 12).

Alterations in serum gastrin levels also failed to influence crypt depth. Jejunal crypt depth increased with age, and with body weight, suggesting that crypt depth is related to one, or both, of these parameters. Where the body weight in 27 day old rats that had undergone rumenectomy was marginally greater than in those animals that had undergone antrectomy or rumenectomy, the crypt depth was also marginally greater. In the colon, both high and low levels of serum gastrin were associated with variable

response in colonic crypt. The hypergastrinaemia associated with fundectomy was, however, associated with a transient increase in the crypt depth at 21 days of age only. This was not reflected in the DNA content of the mucosa at that age.

Although pentagastrin injections have been shown to increase DNA synthesis in the duodenum of rats with low serum gastrin levels, which, in turn, had been induced by starvation (170), and although pentagastrin injections also increase DNA synthesis in the colon in rats with low serum gastrin levels induced by a liquid diet (306), increased DNA content was not observed in association with increased gastrin levels, either in the jejunum or in the colon. To the contrary, an increased serum gastrin level was associated with an apparent drop in jejunal DNA content. Furthermore, a raised serum gastrin level was associated with a low sucrase activity. As delayed increases in disaccharidase activity have also been recorded when the actions of ornithine decarboxylase activity are blocked, which, in turn, inhibits DNA synthesis and intestinal mucosal maturation (233), these results would suggest that disaccharidase activity is delayed in these rats as a secondary phenomenon to decreased DNA synthesis. Again, this study demonstrated accelerated mucosal maturation after surgical stress in the form of an early rise in sucrase activity in those rats undergoing antrectomy or rumenectomy. In this instance, however, these changes were not related to the loss of absorptive surface area, and furthermore, a high serum gastrin level apparently blocked this early increase in activity, and a low serum gastrin level enhanced it.

In this study, the effects of serum gastrin on gastric hyperacidity were not elucidated, but it is possible that alterations in gastric acidity may have been responsible for the observed variations in mucosal maturation, crypt depth, and mucosal DNA content. Nevertheless, many of the changes that were observed in those animals undergoing antrectomy or fundectomy were also present in those animals that had undergone rumenectomy, in which serum gastrin levels were normal.

In addition, after antrectomy, this experiment failed to demonstrate any of the atrophic changes seen in the intestinal tract in other experimental situations, where there is a low serum gastrin level and an absence of normal luminal nutrition (171, 177, 244). Therefore, this study would suggest that the atrophy seen in those situations is the result of the absence of normal luminal nutrition, and not the result of the low serum gastrin. Furthermore, the low serum gastrin in those studies, like the atrophy, would appear to be the result of the absence of food.

The results of this experiment would suggest that exogenous gastrin would be of no value as therapy for short gut syndrome, and this is in agreement with other observations demonstrating that exogenous or endogenous gastrin does not accelerate DNA synthesis after small intestinal resections (256, 308, 375). There may, however, be a role for intraluminal gastrin, but again, only where the mucosa has been made atrophic by parenteral nutrition, or is not normally subjected to high nutrient concentrations (172, 173, 375).

Despite producing elevated serum gastrin levels after fundectomy in this study, there was no effect on the growth or the maturation of the intestine that might be considered beneficial to the newborn animals undergoing intestinal resection. If anything, the delay in the normal rise in the sucrase activity, the decrease in jejunal DNA content (albeit minimal), and the absence of maximal intestinal elongation, would all mitigate against the use of gastrin as an adjunct to treatment of neonatal short bowel syndrome.

CHAPTER EIGHT

SALIVA AND EPIDERMAL GROWTH FACTOR

In the previous experiment, greater than four-fold increases in endogenous serum gastrin levels failed to induce any increase in intestinal growth, or to accelerate intestinal mucosal maturation. As a result, further studies in this direction were not pursued. Nevertheless, other gastrointestinal polypeptides appear to influence intestinal growth and development. One of these, epidermal growth factor (E.G.F.) is present in high concentration in the saliva of male mice (261, 262), in breast milk (62), and in human pancreatic juice (155). In the second study on the effects of gastrointestinal polypeptides, we examined the effects of desalivation on the subsequent intestinal adaptation in male mice.

Although luminal nutrition plays the major role in inducing the intestinal hyperplasia that follows intestinal resection (85, 101, 258, 381), other factors, such as pancreatiko-biliary secretions, play a contributory or permissive role (324, 381, 384). One further secretion that has been shown to influence cellular proliferation in the intestinal tract is saliva, and it could be possible that its effect is mediated via E.G.F. Desalivation has been shown to reduce the DNA and the RNA content of the intestine, and to reduce thymidine incorporation within the intestinal tract of mice when compared with mice undergoing persistent stimulation of saliva secretion (218). Epithelial cells in the jejunum and the ileum of mice have receptors for E.G.F. (275), and E.G.F. stimulates the incorporation of thymidine into DNA throughout the gastrointestinal tract, and increases ornithine decarboxylase activity in the stomach and duodenum of the mouse (7, 102, 315, 316). Furthermore, E.G.F. has one further effect on the suckling rodent, as it induces early intestinal mucosal maturation in mice (245).

In this study, we examined the effects of desalivation on the intestinal adaptation that follows intestinal resection, but this time in the adult male mouse.

MATERIALS AND METHODS

In this study, forty day old male mice were used, and housed in the usual manner. Preliminary studies carrying out intestinal resection in these animals demonstrated that, if mice were fed on chow after submandibular sialadenectomy, then they did not gain weight at the same rate as normal mice. If, however, the mice were fed on a liquid diet (in this case Vivonex-HN), then mice, with and without their submandibular salivary glands, gained weight at the same rate. Furthermore, we also found that mice subjected to intestinal resection and reanastomosis frequently developed an unexplained intestinal obstruction at the anastomosis site, despite an apparently wide open anastomosis, and that this obstruction did not occur when the mice were placed on a liquid diet. Furthermore, the mice would not tolerate greater than 40% intestinal resection without an unacceptable mortality rate and weight loss.

Mice were anaesthetised with intraperitoneal chloral hydrate. The first group (n=47) underwent resection of the middle forty percent of the small bowel, and the remainder were reanastomosed using 6.0 interrupted silk. Twenty-three of these mice then underwent removal of the submandibular salivary glands, and the other twenty-four underwent a sham operation, where the glands were carefully mobilized, while their blood supply and nerve supply were carefully preserved. The submandibular salivary glands were removed in this study, as these have been shown to be the major source of salivary E.G.F. (261, 262). The second group (n=35) underwent sham gut resection with laparotomy alone. Seventeen of these underwent sham sialadenectomy also, and the remaining 18 had their submandibular salivary glands removed.

Ten days later, the mice were sacrificed by exsanguination under ether anaesthesia. The mice received 1.0 μ Ci of tritiated thymidine (6.7 Ci per mM, New England Nuclear) sixty minutes prior to exsanguination. The small intestine was removed and measured in the usual manner, and specimens for biochemical assay were prepared as in the suckling rats. Specimens for biochemical assay were removed from 5-10 cm distal to the ligament of Treitz, and from 5-10 cms proximal to the ileo-caecal junction.

DNA assay was carried out by the method of Scott as modified by Hinrichs (154, 322), and DNA specific activity was measured in the DNA fraction. Quench correction was by an internal standard.

RESULTS

Twenty-eight of the forty-seven mice undergoing intestinal resection, and thirty-two of the thirty-five undergoing laparotomy, survived. After sham laparotomy, mice gained more weight than after small bowel resection (Fig. 8.1). Removal of the submandibular salivary glands did not alter these growth patterns. There was, however, a small, but significantly greater, increase in the length of the proximal intestine after intestinal resection (Fig. 8.2), in those mice that had undergone sialadenectomy as well as the partial enterectomy.

The DNA content of the intestine increased after intestinal resection in both the proximal and the distal intestine (Fig. 8.3). At the same time, the incorporation of tritiated thymidine into DNA was also increased both proximally and distally (Fig. 8.4) and, while there was an apparent decrease in the DNA synthesis in both the jejunum and the ileum of those mice that had undergone sialadenectomy as well as intestinal resection, this difference did not reach significant values.

DISCUSSION

Despite the increases in the mean values of tritiated thymidine incorporation into the intestinal mucosa, submandibular sialadenectomy apparently failed to influence the DNA content of the jejunum or the ileum after small bowel resection in the

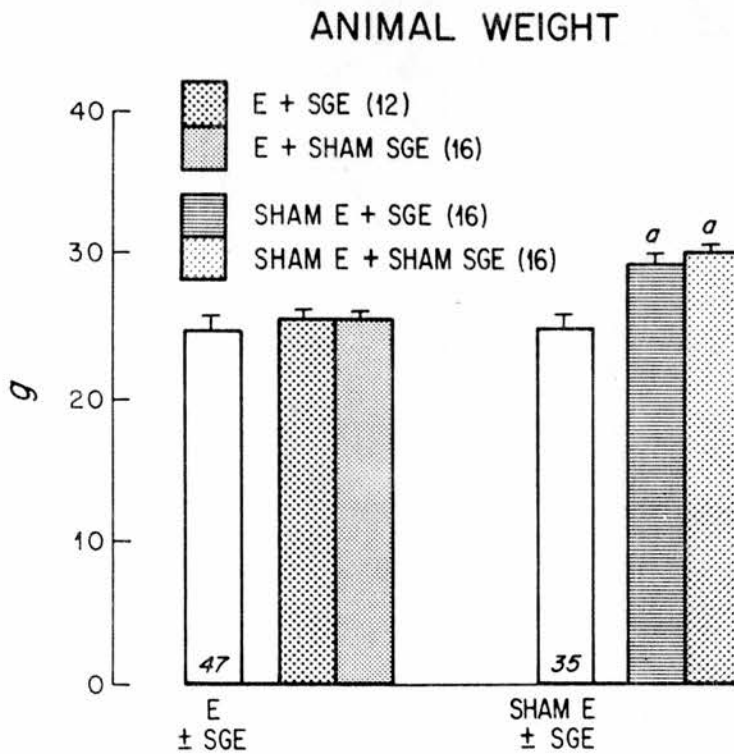


Fig. 8.1 Animal weight (\pm S.E.) at the time of surgery (open bars) and sacrifice (shaded bars). A = $p < .05$ v weight at the time of surgery.

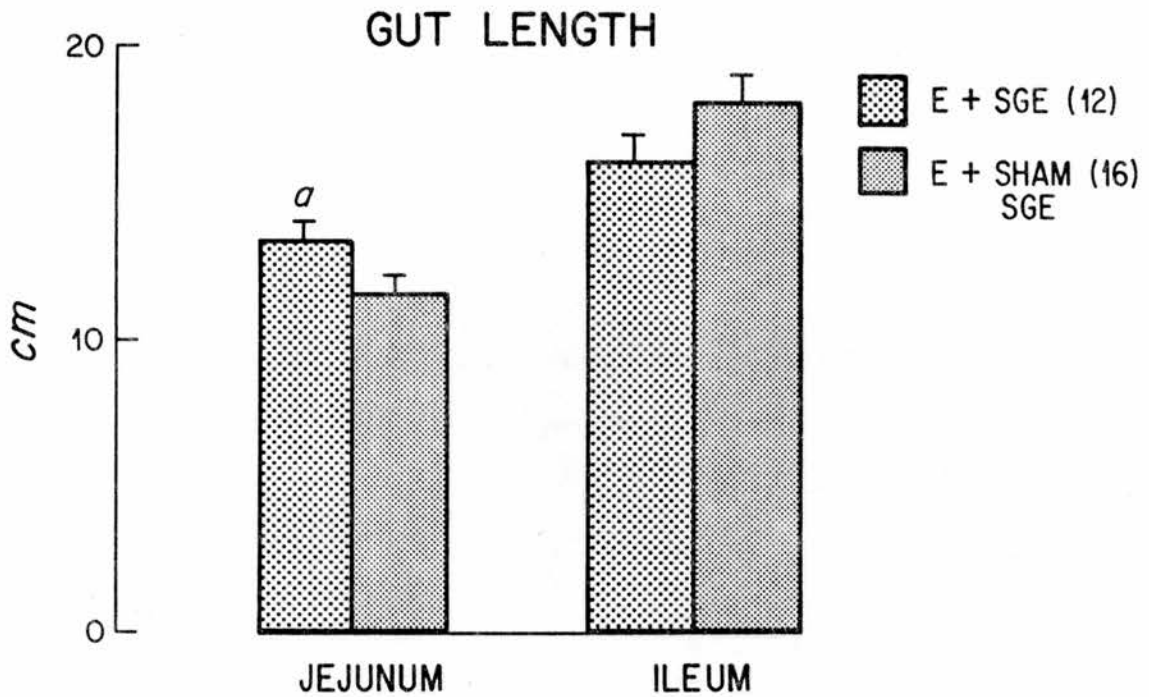


Fig. 8.2 Gut length at the time of sacrifice (\pm S.E.). $A = p < .05$ v animals undergoing enterectomy and sham S.G.E.

male mouse. Therefore, in the absence of a biochemical marker of intestinal hyperplasia, this study would suggest that saliva plays little, if any, role in the intestinal adaptation that normally follows intestinal resection. Possibly E.G.F. produced elsewhere in the intestinal tract (155) obscured the effect of desalivation in this manner. For any future study on the effects of E.G.F. after resection, exogenous E.G.F. will have to be utilized.

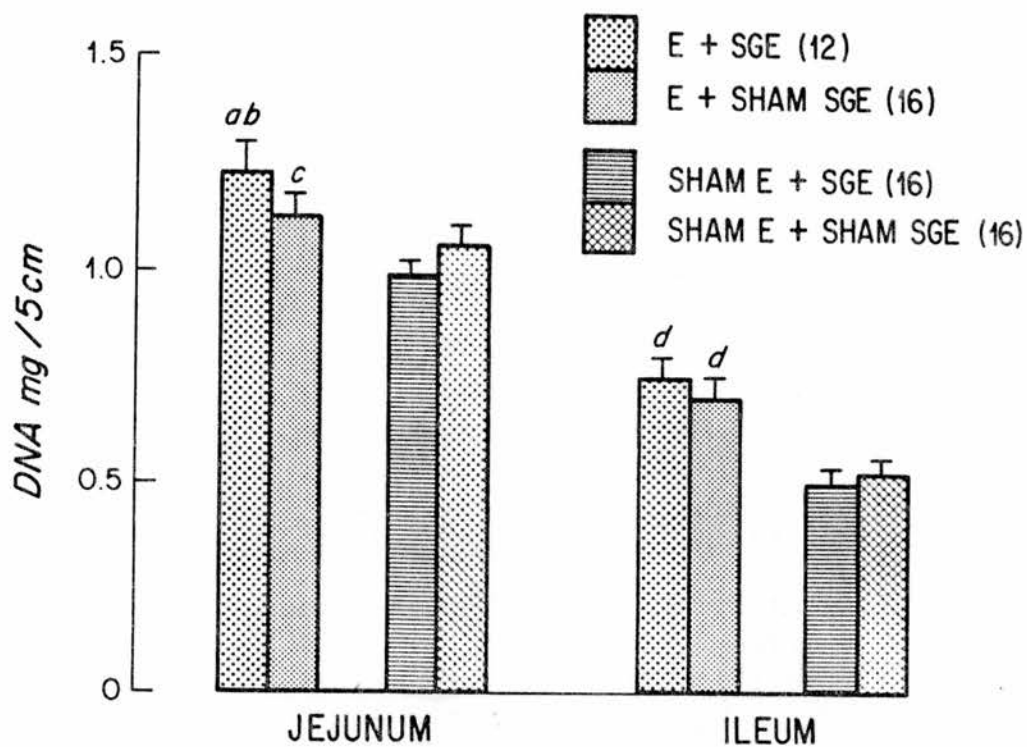


Fig. 8.3 DNA concentration (\pm S.E.). A = $p < .001$ for enterectomy and S.G.E. v sham enterectomy and S.G.E.; B = $p < .001$ for enterectomy and S.G.E. v sham enterectomy and sham S.G.E.; C = $p < .05$ for enterectomy and sham S.G.E. v sham enterectomy and S.G.E.; D = $p < .05$ for enterectomy with S.G.E. or sham S.G.E. v sham enterectomy with S.G.E. or sham S.G.E.

SPECIFIC ACTIVITY

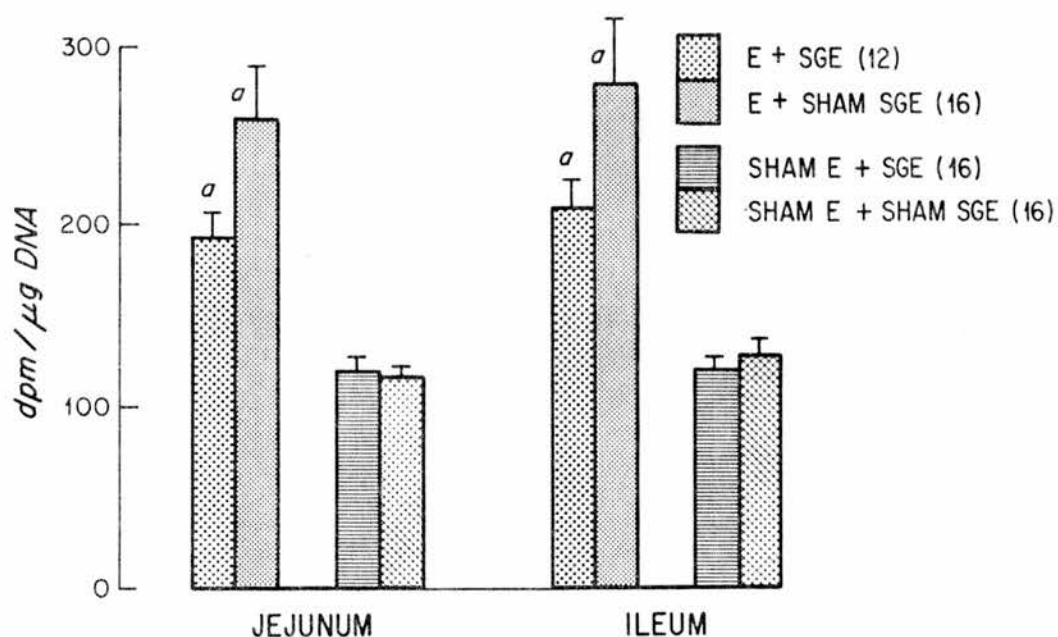


Fig. 8.4 DNA specific activity (\pm S.E.). A = $p < .001$ for enterectomy with S.G.E. or sham S.G.E. v sham enterectomy with S.G.E. or sham S.G.E.

CHAPTER NINE

BREAST MILK

In the third study on growth factors that might promote compensatory regeneration of the intestinal tract after intestinal resection, the effects of breast milk from mothers that had recently given birth was examined. Colostrum and breast milk from females that have recently given birth will stimulate cells to grow in culture (62, 194, 195, 342), and this mitogenic activity would be present within the bowel lumen during the early days of life. Indeed, the small bowel elongates by 45%, and the intestinal mass increases by 260%, when newborn piglets suckle for the first ten days of life (378).

Breast milk mitogen (194, 195, 342), or E.G.F. (62), may be responsible for this stimulus to intestinal growth. Nevertheless, no matter what factor(s) is responsible, the stimulus is specific to the intestinal tract, as isocaloric synthetic feeds fail to produce equivalent intestinal growth, despite equivalent growth of other organs (142). Therefore, 10 day old rats were allowed to suckle, or were gavage fed formula, after partial enterectomy, to determine if intestinal adaptation could be stimulated by early breast milk.

MATERIALS AND METHODS

Litters of 10 day old male Wistar rats (n=250) were housed as previously discussed (Chapters Four and Five). A 60% mid-small-bowel resection was carried out at 10 days of age by removing all but 20% of the proximal and 20% of the distal jejuno-ileum in 80 rats (Fig. 9.1). Intestinal transection was also performed on 80 rats, and a further 80 rats acted as controls. At the beginning of the experiment, ten 10 day old rats were sacrificed, to act as "zero point" controls.

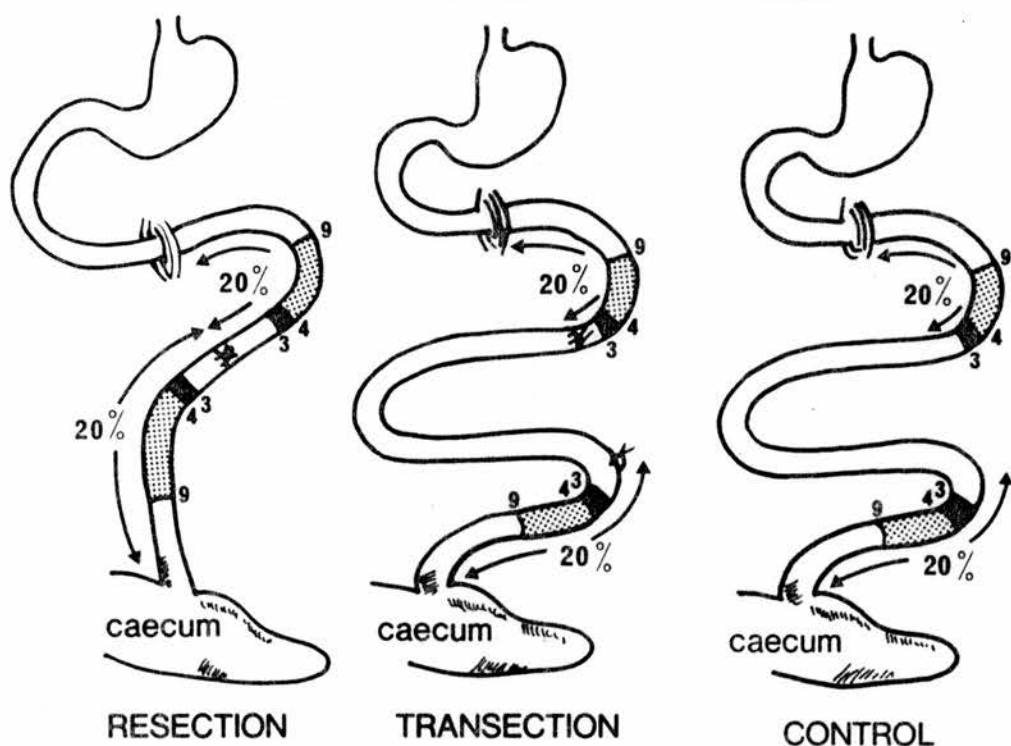


Fig. 9.1 Intestinal resection and intestinal transection. After intestinal resection, 20% of the proximal and 20% of the distal jejunum-ileum remained. The numbers represent the distance in cms from the anastomosis. The stipled area represents the intestine removed for biochemistry, and the solid areas were removed for histology at the time of sacrifice. After intestinal transection, a marking suture was placed at 20% of the length of the jejunum-ileum proximal to the ileo-caecal valve. Equivalent specimens were removed at sacrifice, as after resection. In control animals, 20% of the jejunum-ileum was measured out at sacrifice, and equivalent lengths of intestine removed, as after resection and transection.

Two hours after surgery, one group of 60 pups (20 pups from each group undergoing either intestinal resection, intestinal transection, or no surgery at all) was suckled by foster mothers that had given birth three days previously, so as to receive milk as early as possible in the suckling period; another group of 60 similarly distributed pups was suckled by foster mothers that had given birth ten days previously, so as to receive milk appropriate to their age; a third group of 60 pups was suckled by mothers that had given birth twenty days previously, so as to receive breast milk from late in the suckling period; and a fourth group of 60 pups was fed a commercial formula milk by gavage. If 10 day old rats were fostered to mothers that had only just given birth, then the cannibalism rate was 100%. Handling the foster mothers before delivery (220), or sedating them (259), made no difference to the rate of cannibalism. However, keeping the dam and the litter as quiet as possible (292) appeared to be the most effective way of reducing the cannibalism rate. Even so, the rate was still prohibitively high until dams had been lactating for at least three days. Sixty minutes before pups were fostered to new dams, the natural litter of that dam was removed.

Pups fed the commercial milk formula received Similac S24 LBW (Ross Laboratories, Ohio) at 3-4 hourly intervals, by orogastric gavage with a fine silastic tube. Similac S24 LBW contains 4.5% fat, 8.5% carbohydrate, and 2.2% protein (w/v). After each feed of 3-5 ml, the stomachs, easily seen through the skin in suckling rats, were always full, and at no time during the five post-operative days were the stomachs empty. After each feed, micturition and defaecation were stimulated by stroking the lower abdomen.

After five days of these feeding regimens, all pups were sacrificed by exsanguination under ether anaesthesia. Specimens for histological examination and biochemical examination were removed (Fig. 9.1).

RESULTS

After intestinal surgery, the mortality was 19% (20/160). In control animals that had been fostered, the mortality due to rejection or cannibalism was 22% (18/80). All animals that suckled successfully gained weight (Fig. 9.2).

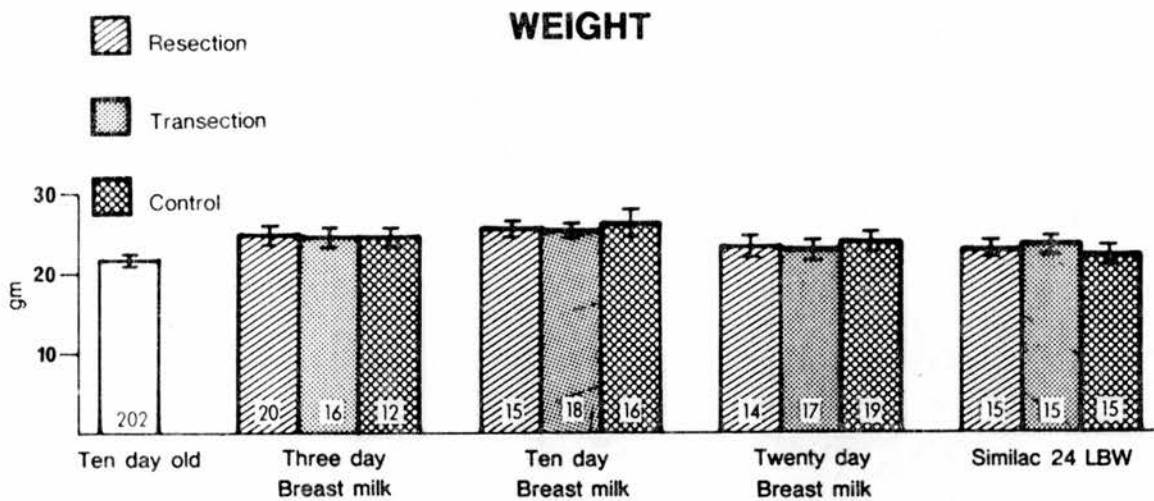


Fig. 9.2 Animal weight (\pm S.E.). There were no differences in the animal weight between the feeding groups at sacrifice.

MUCOSAL MORPHOLOGY

Intestinal Length

Five days after intestinal resection or transection, the lengths of the proximal and distal 20% of the jejuno-ileum were similar in all four feeding groups. In gavage fed rats, however, the proximal 20% of the small intestine was shorter after intestinal resection, or transection, than the proximal 20% of the intestine in control animals (Fig. 9.3).

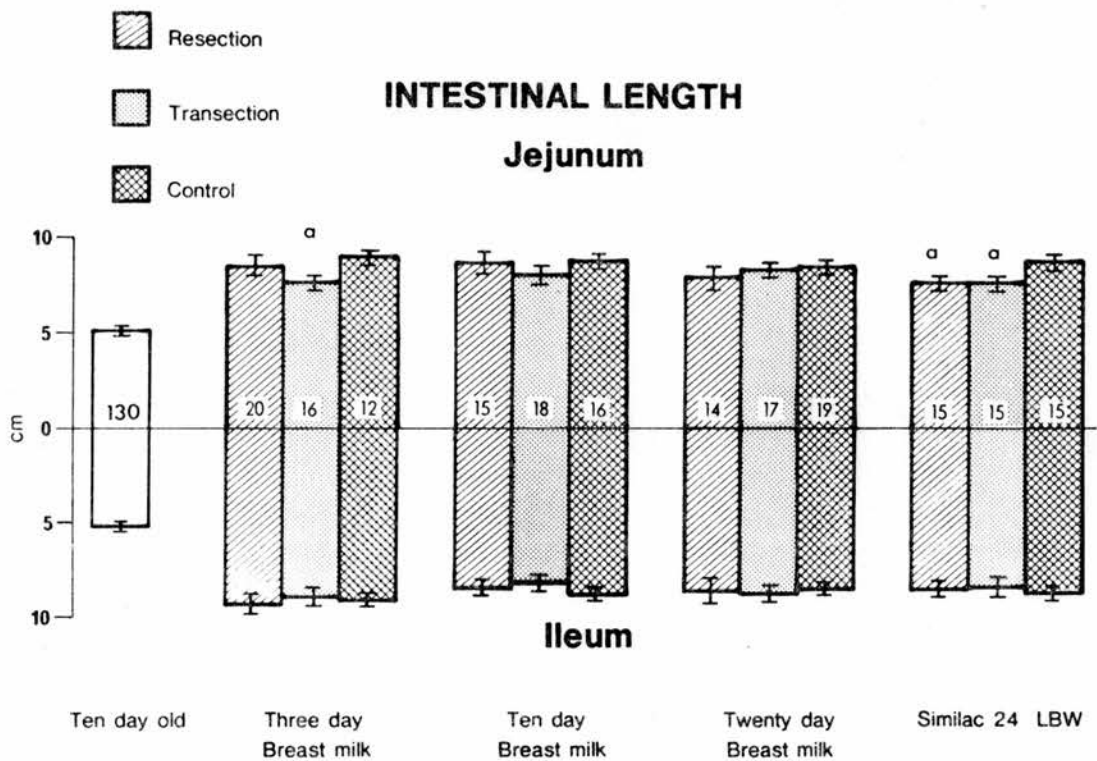


Fig. 9.3 Intestinal length (\pm S.E.). The length of the residual 20% of the intestine is shown at the time of sacrifice. $\alpha = p < .05$ v control.

Villus Height

No matter what the feeding regimen, after intestinal resection, or intestinal transection, the villi were taller than in control animals (Fig. 9.4). Resection produced the taller villi, however. After intestinal resection, the mean increase in the jejunal villus height was 33% when compared with the jejunal villi of normal rats fed in the same manner and, after intestinal transection, the mean increase in jejunal villus height was 26% when compared with the jejunal villi of normal rats fed in the same manner. Distally, there was an even greater response after intestinal resection. The mean increase in ileal villus height was 65% when compared with the ileal villus height in normal animals fed in the same way and, after intestinal transection, the mean increase in the ileal villus height was 22% when compared with normal rats fed in the same way.

Suckling different breast milks modified that response. In the proximal intestine, the villi of those rats that had undergone intestinal resection and then suckled 10 day breast milk were 19% taller than those gavage fed after the same surgery ($p < .02$) and, after intestinal transection, the villi of those rats that were fed the 10 day breast milk were 24% taller than those gavaged after the same surgical procedure ($p < .01$). The only other significant difference in villus heights in the proximal intestine was a significant atrophy of the villus in control rats fed the Similac by gavage ($p < .001$ v 10 day old rats, and those fed 3 day, 10 day and 20 day breast milk for five days).

In the distal intestine, there were more marked differences between the four feeding regimens. After intestinal resection and suckling 3 day breast milk, the distal villus was 28% taller than in gavage fed rats ($p < .001$). The ileal villus was 36% taller than in gavage fed rats when rats suckled 10 day breast milk ($p < .001$), and the ileal villus was 12% taller than in gavage fed rats when rats suckled 20 day breast milk ($p < .05$). Suckling the earlier, as opposed to later breast milks, was also associated

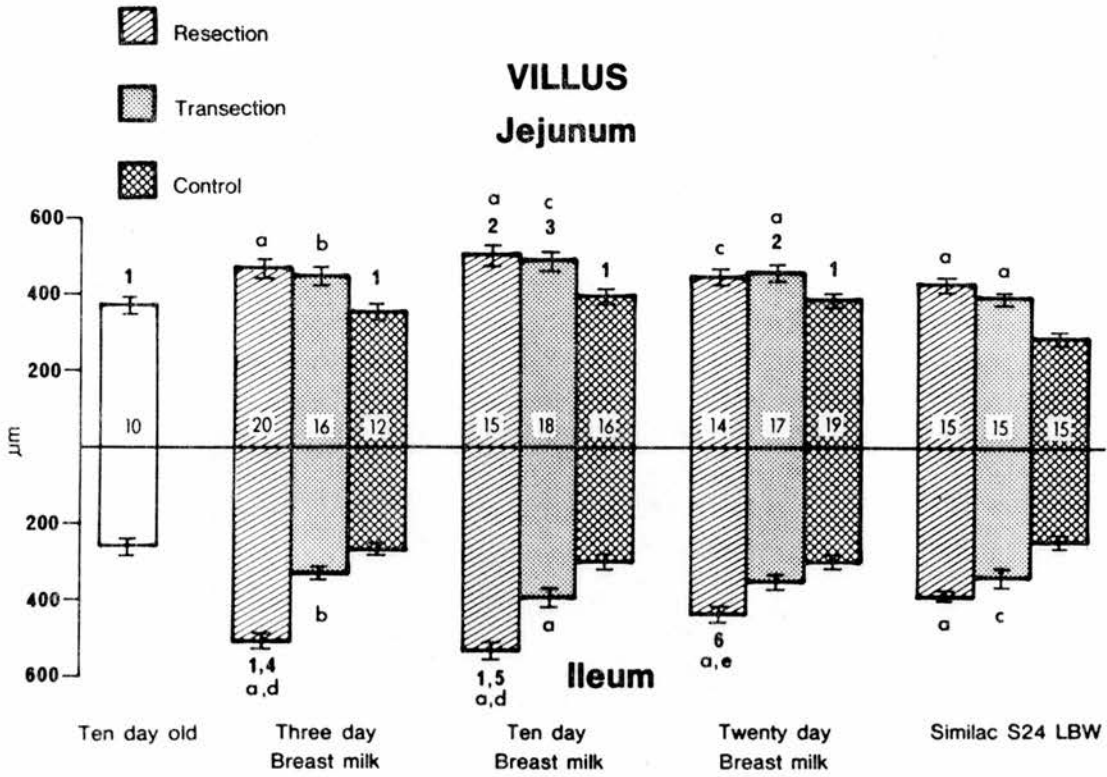


Fig. 9.4 Villus height (\pm S.E.). When comparing animals within the same feeding group, A = $p < .001$ v controls; B = $p < .02$ v controls; C = $p < .01$ v controls; D = $p < .001$ v transection; E = $p < .01$ v transection.

When comparing animals within the same surgical group but fed different milks, 1 = $p < .001$ v formula; 2 = $p < .02$ v formula; 3 = $p < .01$ v formula; 4 = $p < .05$ v 20 day breast milk; 5 = $p < .01$ v 20 day breast milk; 6 = $p < .05$ v formula.

with an increased villus height. The ileal villus of the rats suckling the 3 day breast milk after intestinal resection was 14% taller than rats suckling the 20 day breast milk ($p < .05$), and the ileal villi of the rats suckling the 10 day breast milk after intestinal resection was 22% taller than rats suckling the 20 day breast milk ($p < .01$). After intestinal transection, there was no difference in villus height between feeding groups. Furthermore, there was no villus atrophy in the ileum of normal rats fed by gavage.

Crypt Depth

No matter what the feeding regimen, five days after intestinal resection, or intestinal transection, the crypts increased in depth (Fig. 9.5). The crypts were significantly deeper after resection than after transection. After resection, the mean jejunal crypt depth was 82% greater than in the jejunum of normal rats fed

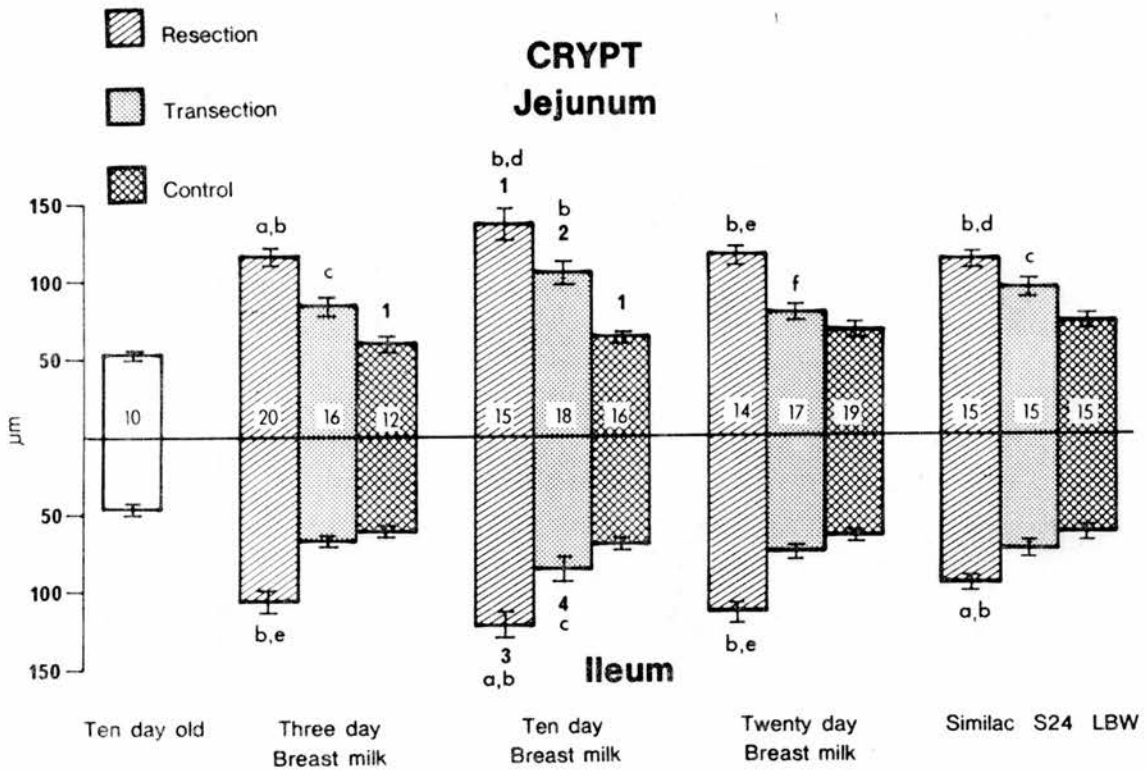


Fig. 9.5 Crypt depth (\pm S.E.). When comparing animals within the same feeding group, A = $p < .01$ v transection; B = $p < .001$ v controls; C = $p < .02$ v controls; D = $p < .05$ v transection; E = $p < .001$ v transection; F = $p < .05$ v controls.

When comparing animals within the same surgical group but fed different milks, 1 = $p < .05$ v formula; 2 = $p < .01$ v 20 day breast milk; 3 = $p < .02$ v formula; 4 = $p < .02$ v 3 day breast milk.

in the same manner and, after transection, the mean jejunal crypt depth was 39% greater than in rats fed in the same manner. Distally, the ileal crypts became, on average, 70% deeper after intestinal resection, but after intestinal transection the increase in ileal crypt depth was minimal.

Feeding different breast milks again modified the response to surgery. In the proximal intestine, when rat pups suckled 10 day breast milk after intestinal resection the crypts were 21% deeper than in those pups fed by gavage ($p < .05$) and, after intestinal transection, when rats were also fed the 10 day breast milk, the crypts were 9% deeper than in pups fed by gavage ($p < .02$). The crypts in the control animals fed on 10 day and 3 day breast milk were, however, smaller than in those fed by gavage ($p < .05$ v those fed 3 day and 10 day breast milk).

In the distal intestine, the crypts were 27% deeper after intestinal resection if pups were fed 10 day breast milk rather than being gavage fed ($p < .01$) and, after intestinal transection, the crypts were 30% deeper if pups were fed 10 day breast milk rather than suckling 3 day breast milk ($p < .02$).

BIOCHEMICAL ASSAYS

Mucosal DNA Content

There was cellular hyperplasia in the residual intestine after intestinal resection, no matter what the feeding regimen. The response was recorded both proximal and distal to the intestinal resection, but was most marked distally (Fig. 9.6). After intestinal transection, a less marked, but nonetheless significant, response was also observed. Again, the DNA content was modified by the feeding regimen that followed the intestinal surgery. In the proximal intestine five days after an intestinal resection, the DNA content was 17% less after feeding 3 day breast milk than after feeding 20 day breast milk ($p < .001$) and, after feeding 10 day breast milk, was 16% less than after feeding 20 day breast milk

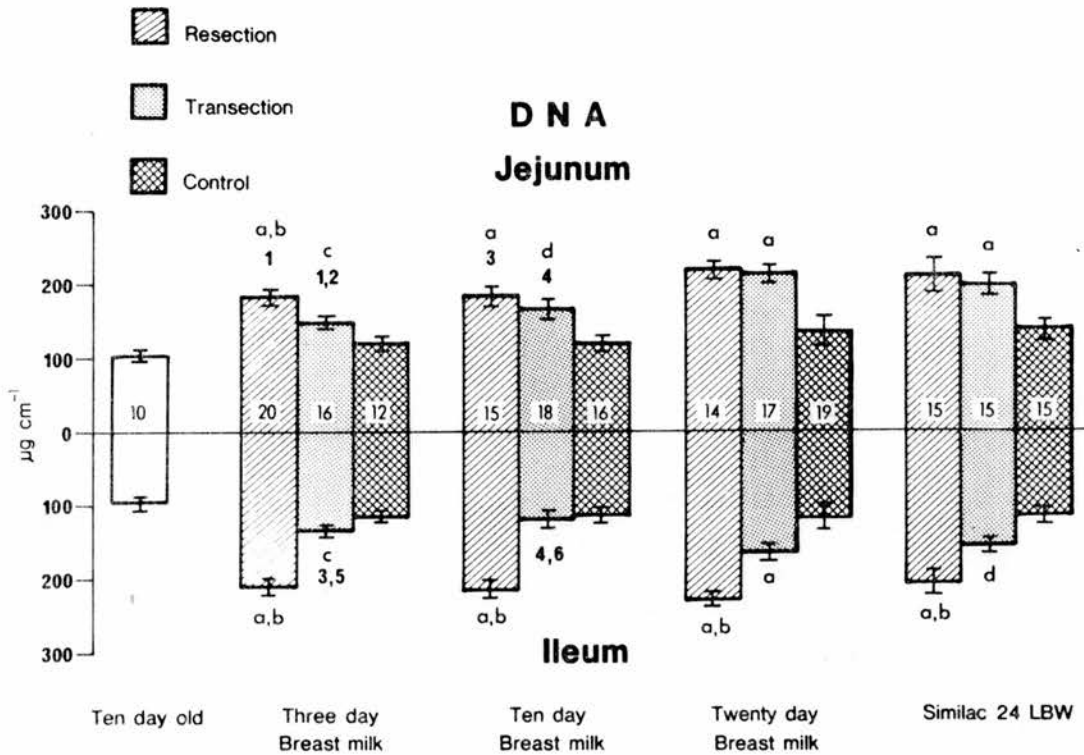


Fig. 9.6 DNA content (\pm S.E.). When comparing animals within the same feeding group, A = $p < .001$ v controls; B = $p < .001$ v transection; C = $p < .02$ v controls; D = $p < .01$ v controls.

When comparing the animals within the same surgical group but fed different milks, 1 = $p < .001$ v 20 day breast milk; 2 = $p < .001$ v formula; 3 = $p < .02$ v 20 day breast milk; 4 = $p < .01$ v 20 day breast milk; 5 = $p < .05$ v formula; 6 = $p < .02$ v formula.

($p < .02$). After intestinal transection, when pups were fed the 3 day breast milk, the DNA content of the proximal intestine was 32% less than after feeding 20 day breast milk ($p < .001$), and 31% less than after gavage ($p < .001$). When pups were fed the 10 day breast milk after intestinal transection, the DNA content in the proximal intestine was 24% less than after feeding the 20 day breast milk ($p < .01$).

When pups suckled 3 day breast milk after intestinal transection, the DNA content in the distal intestine was 14% less than when pups were gavaged ($p < .05$), and 18% less than when pups suckled 20 day breast milk ($p < .02$). When pups suckled 10 day breast milk, the DNA content of the distal intestine was 27% less than when they suckled 20 day breast milk ($p < .01$), and 24% less than when they were gavaged ($p < .02$).

Sucrase Activity Per mg DNA

In the normal 10 day old rat, sucrase activity was barely detectable but, only five days after intestinal resection, the sucrase activity had risen sharply, both proximally and distally (Fig. 9.7). After gavage feeding, the sucrase activity was greater in all three surgical groups.

In the jejunum, the mean sucrase activity five days after an intestinal resection was six times greater than in normal rats fed in the same manner. When rats from similar surgical groups were compared, however, there was no significant difference between the four feeding regimens. After intestinal transection, the jejunal sucrase activity per mg DNA was no greater than in normal rats fed in the same way for the five day period, and, again, there was no difference between feeding regimens.

After intestinal resection, the mean sucrase activity per mg DNA in the ileum was five times that of control animals. In contrast to the lack of response in the jejunum, however, the feeding of early breast milk was associated with greater sucrase activity per mg DNA in the ileum than the feeding of a breast milk from later in the suckling period. However, gavage feeding of a diet containing a high carbohydrate load induced the greatest mucosal sucrase activity after intestinal resection.

Sucrase Activity Per Centimeter

After intestinal resection, both the sucrase activity and the DNA content of the residual intestine rose. As a result, the sucrase activity per centimeter rose markedly after intestinal resection (Fig. 9.8). Proximal to the intestinal anastomosis, no differences were seen between the three groups of breast-fed rats, but distal to the anastomosis the feeding of early breast milk after intestinal resection was associated with small increments in sucrase activity. Again, the introduction of a high carbo-

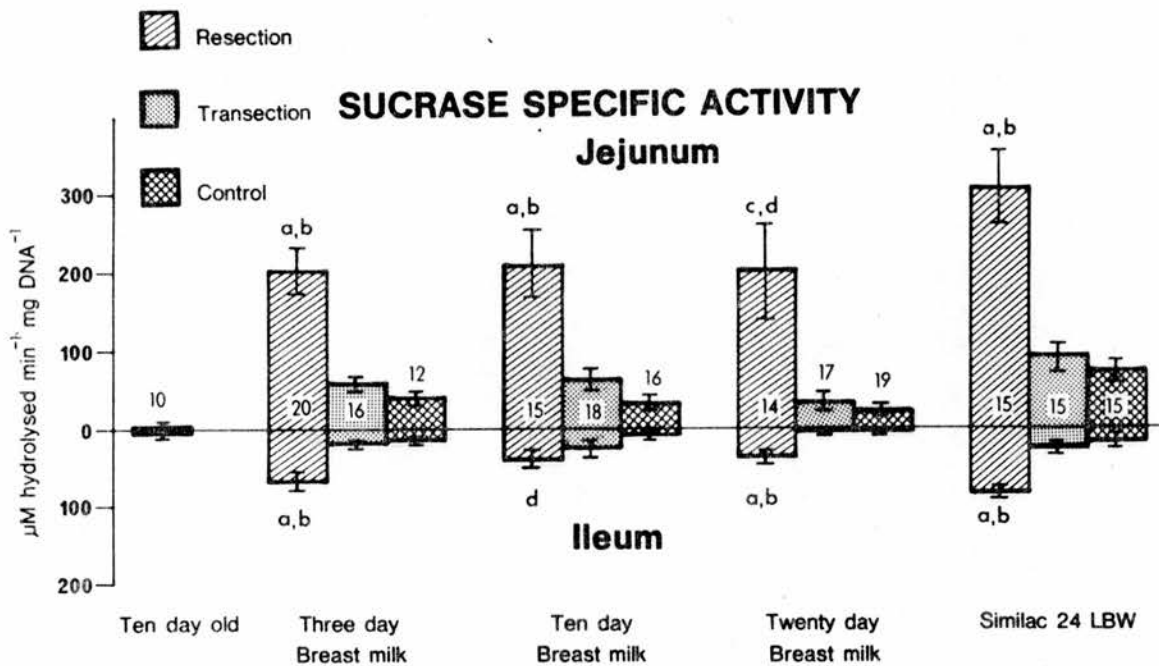


Fig. 9.7 Sucrase specific activity per mg DNA in the residual small intestine (\pm S.E.). A = $p < .001$ v transection; B = $p < .001$ v controls; C = $p < .01$ v transection; D = $p < .01$ v controls.

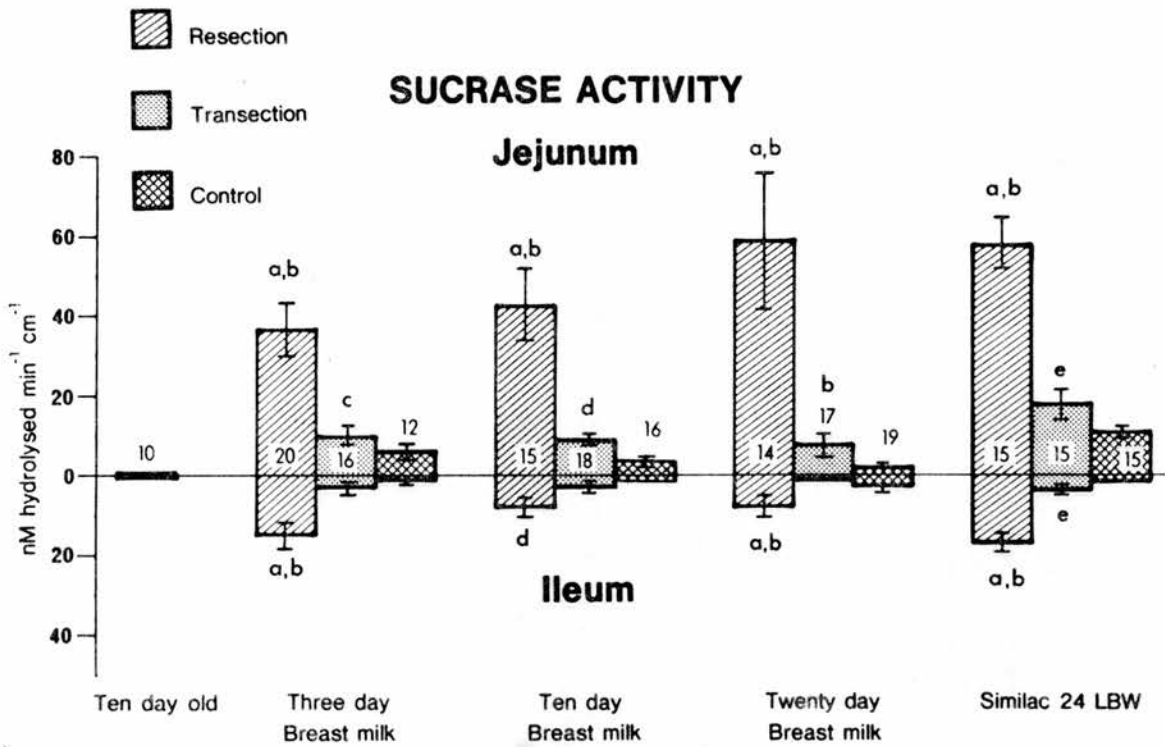


Fig. 9.8 Sucrase activity (\pm S.E.). A = $p < .001$ v transection; B = $p < .001$ v controls; C = $p < .05$ v controls; D = $p < .01$ v controls; E = $p < .02$ v controls.

hydrate diet by gavage produced the greatest sucrase activity per centimeter.

DISCUSSION

Despite the slow cell production and cell turnover, and despite the negligible sucrase activity in the intestinal mucosa of the ten day old suckling rat (11, 147, 255, 339), intestinal resection induces a rapid expansion of the cell population, an increase in villus height and crypt depth, and an increase in mucosal sucrase activity. Furthermore, breast milk enhances the villus growth that occurs in response to an intestinal resection.

When suckling rats undergo intestinal resection, breast milk from mothers that have been lactating for only three days stimulates greater villus growth than breast milk from mothers that have been lactating for twenty days. Nevertheless, both of these breast milks stimulate greater villus growth than that produced by commercial formula milk.

Luminal nutrition is one of the major stimuli to intestinal adaptation after intestinal resection (85, 381). By removing the intervening small bowel, distal intestine is brought into contact with higher concentrations of the products of the initial phases of luminal digestion that are normally only found in the proximal small intestine (9, 12, 85). As a result, the stimulus to intestinal adaptation would be minimal proximally, and most marked distally (85, 381).

Therefore, if breast milk, and especially early breast milk, contained a factor(s) that could stimulate increased intestinal compensation in excess of the usual adaptation to intestinal resection, that response would be expected to be most marked distally. Furthermore, as this factor(s) stimulates normal mucosal growth in suckling animals (62, 131, 142, 320, 378), then any reduction in normal growth would be most marked proximally when that stimulus is removed.

Earlier work has demonstrated that breast milk produces a 45% increase in small bowel length, and a 260% increase in small bowel mass, when piglets are suckled for ten days (378). When neonatal puppies suckled colostrum, or were fed a synthetic isocaloric substitute, the mucosal DNA was 60% greater, the mucosal mass was 82% greater, and the mucosal protein content was 91% greater in those animals that suckled than in those animals that received the synthetic milk mixture (142). One possible explanation of this dramatic increase in the mucosal mass and protein is that intact proteins are taken up by the intestinal mucosa (131, 259, 378), but this could not explain the increased DNA content (142).

Bovine, ovine and human colostrum, and early breast milk, contain mitogenic activity (194, 195, 342). Furthermore, the mitogenic activity is greatest in colostrum, and then diminishes rapidly as lactation in ovine, bovine and human breast milks progresses (194, 195, 320, 349). Both the acid stable breast milk mitogen, a polypeptide (14,000-18,000 Daltons) that passes through the stomach intact (194, 195, 349), and epidermal growth factor (62), another polypeptide (E.G.F. = 74,000 Daltons as the inactive complex, and 6,045 Daltons as the active sub-unit) (261, 262), have been found in breast milk (62, 349). Colostrum and breast milk from lactating sheep, cows and humans (62, 194, 195) stimulate epithelial cells and Balb/c 3T3 cells to grow in culture, and anti-E.G.F. gamma-globulin inhibits 93% of the stimulatory effect of human breast milk (62).

E.G.F. has been shown to stimulate DNA synthesis (7, 63, 79, 80), to regulate the normal circadian rhythm of DNA synthesis (315, 316), and to increase ornithine decarboxylase activity within the gastrointestinal tract (102). Furthermore, submandibular sialadenectomy in male mice has been shown to decrease mucosal DNA synthesis and content within the intestinal tract (218). As the submandibular salivary gland in the male mouse contains abundant E.G.F. (261, 262), E.G.F. is implicated in the regulation of intestinal mucosal growth (79), especially as receptors for E.G.F. are present throughout the small intestine of rats (109).

Whatever the explanation of the increased intestinal growth when animals are fed colostrum, colostrum and early breast milk contain a factor(s) that can induce intestinal growth in the early days of life. If this mitogenic activity was the reason for the increased villus height after intestinal resection and the feeding of early breast milk, however, then the DNA content of the intestinal mucosa should also have risen at the same time. It did not.

As the suckling rat, unlike the adult rat, responds to intestinal resection with cellular hypertrophy, as well as cellular hyperplasia (see Chapter Ten), the increased villus height after

the feeding of breast milk after intestinal resection, as opposed to the feeding of formula, appears to be the result of an increased cell size in the breast-fed groups. As an increase in mucosal cell size occurs in the mucosa of the normal suckling rat as the intestine matures and the rat weans (201), as suckling breast milk as opposed to gavaging a similar synthetic feed to suckling rabbits is associated with an early maturation of the intestinal mucosa (358), and as an increase in the RNA:DNA ratio was seen in the same age of rat after intestinal resection (see Chapter Ten), the combined effect of intestinal resection and the feeding of early breast milk could well stimulate early mucosal maturation, and an associated increase in cell size, greater than either one alone. Then, this increased size of the individual enterocyte might explain the increased villus height in the absence of increased mucosal DNA content.

Apart from the rapid structural changes that occurred after intestinal resection in suckling rats, intestinal sucrase activity, proximal and distal to the intestinal anastomosis, also increased five days after intestinal resection, no matter what the feeding regimen. Although such an increase in sucrase activity could be interpreted as an adaptational response to intestinal resection (56, 381), this is difficult to explain when there is no sucrose in rat breast milk (123, 130, 147). There are, however, two aspects to this response. Proximally, luminal nutrition is unlikely to increase after intestinal resection in suckling rats, as the volume of milk ingested is determined by the dam, and not by the pup (147, 148). Therefore, hyperphagia could not explain the increased proximal sucrase activity (250) when rats were fed by dams that had only been lactating for three days before the fostering, especially as the dam that had been feeding the 20 day old rats would have been producing the greatest volume of milk (147). Therefore, a systemic enterotropic response to intestinal resection must be invoked, to explain this proximal response (347, 383). This systemic response, like stress elsewhere in the suckling rat, would bring

about an increase in the sucrase activity in the proximal small intestine (359). Similar precocious increases in jejunal sucrase activity have been observed after other stresses to the suckling rat pup, notably forced weaning (43), gastrostomy (211), or ileal bypass (Chapter Two). Furthermore, similar increases in jejunal sucrase activity were observed in association with precocious maturation of mucosal barrier function and increases in cell size after intestinal resection in 10 day old suckling rats (see Chapter Ten). After intestinal resection, suckling breast milk, as opposed to being fed by gavage, did not alter the sucrase activity per centimeter in the proximal small bowel.

In the distal small intestine, increased luminal nutrition occurs after an intestinal resection (85). As a result, the increased carbohydrate load reaching the distal small bowel could induce increased sucrase activity even in the absence of sucrose in the diet (56, 85, 149, 255). Therefore, the summation of the systemic effects of intestinal resection, the possible effects of E.G.F., and the increased luminal nutrition, might explain the more pronounced increase in sucrase activity seen in the distal intestine when rats were fed early breast milk after intestinal resection.

The increase in the ileal sucrase activity after suckling the earlier breast milks after intestinal resection may also reflect the increased ileal villus height in these rat pups. When enterocytes migrate from crypt to villus tip, the sucrase activity increases as they do so (44; see Chapter Four). As a result, the taller villi would be expected to have more sucrase activity, and the earlier breast milks may also induce a greater sucrase activity in the intestinal mucosa by this mechanism.

When rats were gavage fed a diet containing 8.5% carbohydrate (as 4.25% lactose and 4.25% polycose, Ross Laboratories, Columbus, Ohio), sucrase activity per mg DNA was greater than after suckling breast milk, which has less than half the carbohydrate load (3.3% lactose) (12, 147). In the suckling rat, sucrase activity can be

induced by stress alone (211, 359), or by a high carbohydrate diet alone (149, 255), but the two acting synergistically would produce an even greater response (255). Therefore, it is possible to speculate that, in suckling animals, the stress of intestinal surgery, the presence of a breast milk mitogen, and the presence of a high carbohydrate load, all need to be present in the distal small intestine to produce the maximal functional adaptational response to intestinal resection.

SECTION FOUR

ANTIGEN TRANSPORT

CHAPTER TEN

BOVINE SERUM ALBUMIN

One of the normal characteristics of intestinal mucosal maturation is the increasing exclusion of antigenic macromolecules that occurs at the time of weaning (77, 147, 212, 364, 365). At the same time, the well recognized changes in mucosal enzyme activity (147, 255), crypt depth (151), villus height (151), cellular RNA:DNA ratios (151), and cell kinetics occur (202). As the stress of simple gastrostomy, or the stress of premature weaning, can both induce many of these changes precociously (43, 212), intestinal surgery might also produce an early induction of antigen exclusion as part of that early mucosal maturation.

On the other hand, in mature animals, when the intestine is damaged by surgical trauma (294), generalized mucosal injury (75, 293, 343), malnutrition (366, 396), or infection (31, 366), excess

antigen penetration of the mucosal barrier occurs (366). Furthermore, intestinal resection produces mucosal hyperplasia which, in turn, may increase the number of potential sites for antigen transport. Therefore, the combined effects of intestinal damage and mucosal hyperplasia might increase antigen absorption through the mucosa of the residual intestine when part of the small bowel has been removed (Fig. 10.1).

MATERIALS AND METHODS

Litters of 10 day old male Wistar rats ($n=145$), 21.1 ± 0.2 g, were subjected to a 60% mid-small-bowel resection ($n=60$), intestinal transection ($n=30$), or no surgery at all ($n=55$), as in Chapter Nine.

To determine the most appropriate time for sacrifice of rats after administering BSA, two preliminary studies were carried out. The first to determine the length of time it would take for the whole of the small intestine to be exposed to BSA, and the second to determine how soon after gavage peak levels of serum BSA occurred.

1. Intestinal transit time

Intestinal transit was examined at 15 days of age in 5 normal rats, in 5 rats that had undergone intestinal resection, and in 5 rats that had undergone intestinal transection. In this preliminary study, rats were gavage fed a 1.0 ml suspension of powdered charcoal, which is plainly visible through the intestinal wall of these suckling animals. Gavage of this charcoal, and the BSA in the subsequent BSA studies, was carried out by introducing an 0.030 inch I.D. sialastic tube (Dow Corning) into the stomach of each animal, while awake. All animals were fasted for 12 hours before gavage. After gavaging the charcoal, one animal from each group was sacrificed at thirty minute intervals, until charcoal could be seen at the ileo-caecal valve.

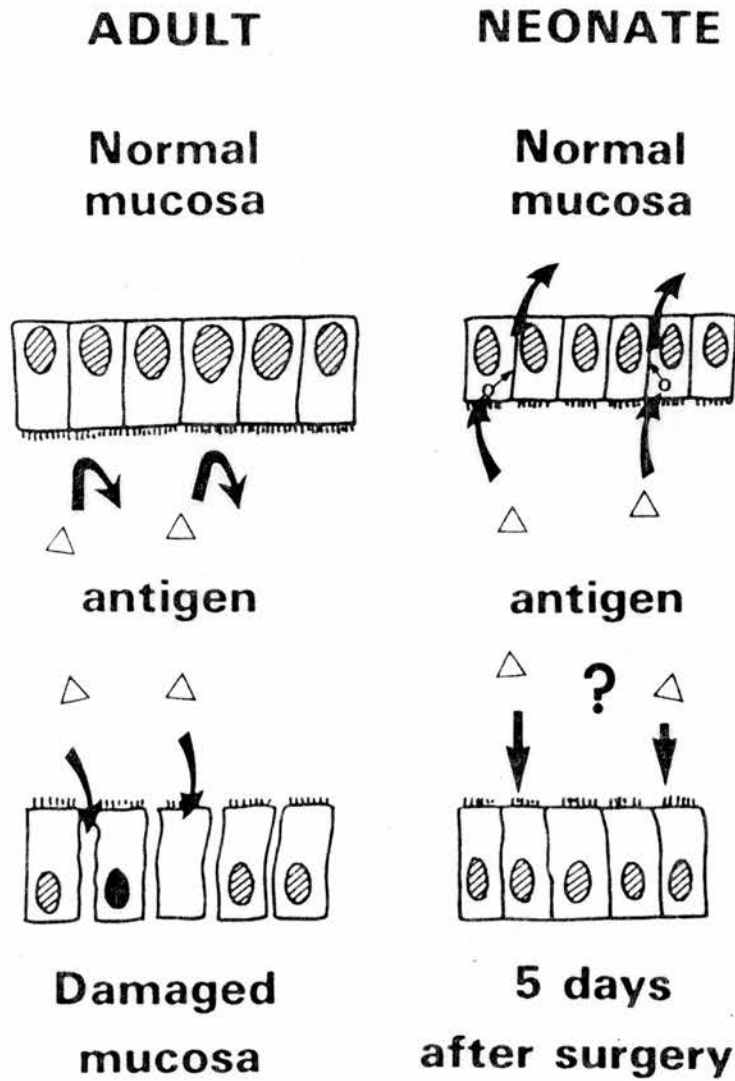


Fig. 10.1 Adult and neonatal handling of enterically presented antigen. Adult: intestinal damage allows antigen between the damaged cells, whereas cell death allows antigen to get into the cell. Neonate: the small immature cell transports antigen across the cytoplasm to the intercellular space from where it can gain access to the circulation.

2. Peak serum levels

To determine the time it took for serum levels of BSA to reach their peak after intragastric gavage at 15 days of age, thirty fasted animals that had undergone intestinal resection, and thirty fasted controls, were gavage fed 100 mg of purified BSA (Sigma). Starting 30 minutes later, five animals from each group were exsanguinated at hourly intervals for 5 hours. Serum was stored at -20°C until levels were quantitated by electro-immunodiffusion studies (209).

The remaining rat pups ($n=18$ after resection, $n=13$ after transection, and $n=17$ controls) were gavage fed 100 mg of purified BSA at 15 days of age. 2.5 hours later, they were exsanguinated under ether anaesthesia, and the serum was stored.

Morphological Studies

The length of the residual proximal and distal intestine was measured at the time of sacrifice, as in the previous studies.

Histological Studies

One centimeter specimens for histological examination were removed from 3-4 centimeters proximal and 3-4 centimeters distal to the anastomosis in those rats that had undergone intestinal resection, and at 3-4 centimeters proximal to the anastomosis and 3-4 centimeters distal to the marking suture in those animals that had undergone intestinal transection. Corresponding sections were taken from control animals. Estimations of crypt depth and villus height were carried out, as in the previous studies. The presence or the absence of supranuclear vacuolation in the mucosal enterocyte was graded as mild, moderate or marked.

Biochemical Studies

The concentration of bovine serum albumin in rat serum was measured by an electroimmunodiffusion technique using rat serum containing known concentrations of BSA as standards (209). After removal of the histological specimens, the remainder of the proximal intestine, the whole of the intermediate intestine in those animals undergoing intestinal transection and control animals, and the remainder of the distal intestine, were rapidly frozen, and then stored at -20°C for biochemical assay (see Appendix One).

Student's *t* test was used for statistical analysis for all results except those relating to the serum BSA levels. For these, Wilcoxon's rank sums test was used to determine whether or not the compared samples came from rats of the same or different populations with respect to the distribution of serum BSA levels.

As the depressed levels of BSA in rat serum might simply reflect the loss of absorptive surface area after intestinal resection, the serum levels of BSA were compared, first to the residual length of the intestine, and then indirectly to the cell population of the residual intestine, by relating the serum BSA levels to the intestinal DNA content, as the DNA content per cell remains a constant (362).

RESULTS

Fifty-three of the 60 animals undergoing intestinal resection suckled successfully, and gained weight after surgery. Three of these 53 animals aspirated and died in the initial transit time study. Eighteen of the 30 rats undergoing intestinal transection survived, and 3 of the 55 control animals were rejected by their dams. There was no difference in the weights of animals undergoing intestinal surgery and normal rats at the time of sacrifice (Table 10.1).

Table 10.1 Weight g (\pm S.E.)

Ten day old	n = 48	21.1 \pm 0.2
Resection	n = 18	27.1 \pm 0.5
Transection	n = 13	28.6 \pm 0.8
Control	n = 17	29.2 \pm 1.0

Transit Time

After gavage, the charcoal suspension took 2.5 hours to reach the ileo-caecal valve in those animals that had undergone intestinal resection. Charcoal was well down into the ileum at that time in control animals, and those that had undergone intestinal transection.

BSA Absorption

1. Peak levels

Peak serum levels of immunoreactive BSA occurred 1.5 hours after intragastric gavage in rats that had undergone intestinal resection, but after 4.5 hours in control rats (Fig. 10.2). After both intestinal resection, and in normal animals, the serum levels of BSA rose for the first 90 minutes. BSA absorption then plateaued for 2 hours in normal rats, but fell after 90 minutes in those undergoing intestinal resection. BSA was almost undetectable by 210 minutes in those rats that had undergone intestinal resection. The serum BSA levels in normal rats climbed to a further peak at 4.5 hours after gavage, and then started to fall.

Since the BSA levels in those rats undergoing intestinal resection was beginning to fall at 2.5 hours after gavage, and as the powdered charcoal entered the colon at 2.5 hours, the definitive study on BSA absorption after intestinal resection, transection, or in control animals, was carried out 2.5 hours after BSA gavage. Furthermore, in this way, any effects due to colonic absorption of antigenic peptides (8, 73) would be avoided.

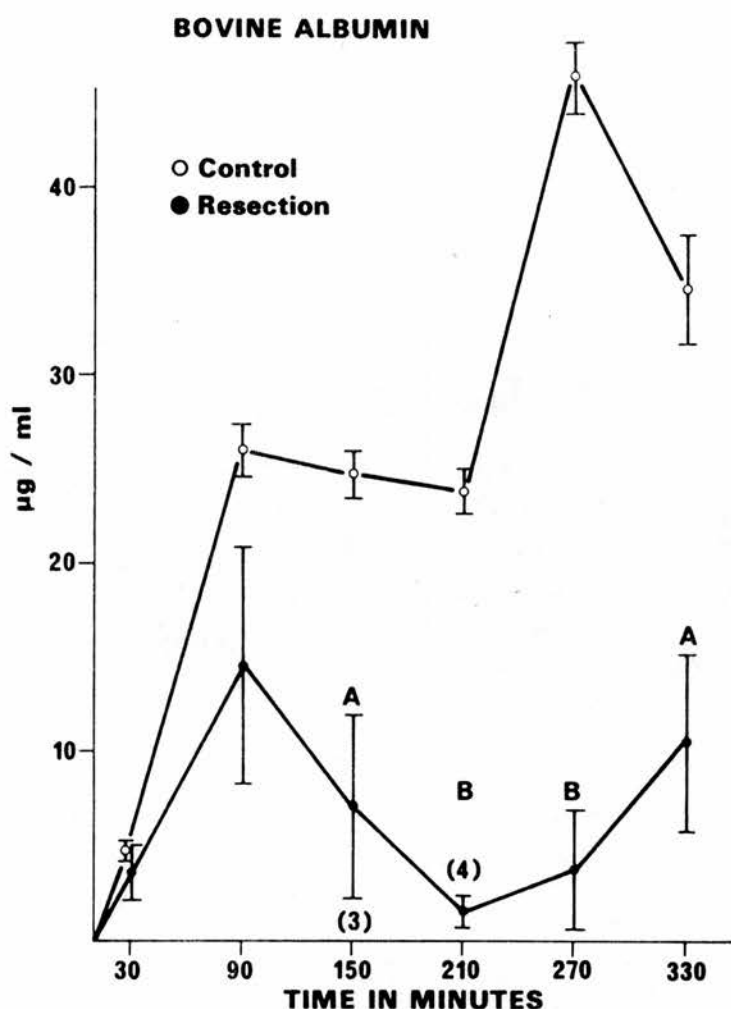


Fig. 10.2 Serum levels of BSA (\pm S.E.) in normal rats and rats that have undergone a 60% mid-small bowel resection for the 5.5 hours following gavage of 100 mg of purified BSA. A = $p < .02$ v controls; B = $p < .03$ v controls (Wilcoxon's rank sums test). Each time point represents 5 rats, unless otherwise specified by the numbers in parentheses.

2. BSA levels in the definitive study

2.5 hours after gavage, the serum levels of BSA in those rats undergoing intestinal resection or transection were lower than in control rats (Table 10.2). After intestinal resection, serum levels of BSA were only one sixth of those in controls and, even when the levels were compared with the length of the residual intestine (Fig. 10.3), or its DNA content (Fig. 10.4), the serum levels of BSA were only one third and one fifth of normal values. After intestinal transection, the serum levels of BSA were one half of those in controls and, when compared with the length of the residual intestine or its DNA content, were again one half of those of controls.

Table 10.2 Rat serum levels of BSA $\mu\text{g/ml}$ ($\pm\text{S.E.}$)

Resection	n = 18	2.96 ± 0.78^a
Transection	n = 13	9.74 ± 2.94^a
Control	n = 17	18.62 ± 1.36

a = $p < .05$ v control rats

Morphological Studies

Intestinal lengths

In comparison with control rats, the jejunum was the same length after intestinal resection, and the ileum had increased by 9% (Table 10.3). After intestinal transection, however, the jejunum was 9%, and the ileum 7%, shorter than in control animals.

Histology

Villus height

After intestinal resection or transection, the villus height and the crypt depth increased (Fig. 10.5). In comparison with control rats, the mean villus height increased by 30% in the jejunum and by 50% in the ileum after intestinal resection, and by 34% in the jejunum and by 5% in the ileum after transection.

Crypt depth

In comparison with control rats, the mean crypt depth increased by 70% in the jejunum and by 55% in the ileum after resection, and by 42% in the jejunum and by 37% in the ileum after transection.

Vacuolation

Enterocyte vacuolation was unaffected by the type of surgery performed (Table 10.4), and had a normal distribution with more marked vacuolation in the distal intestine (325). Surgery did

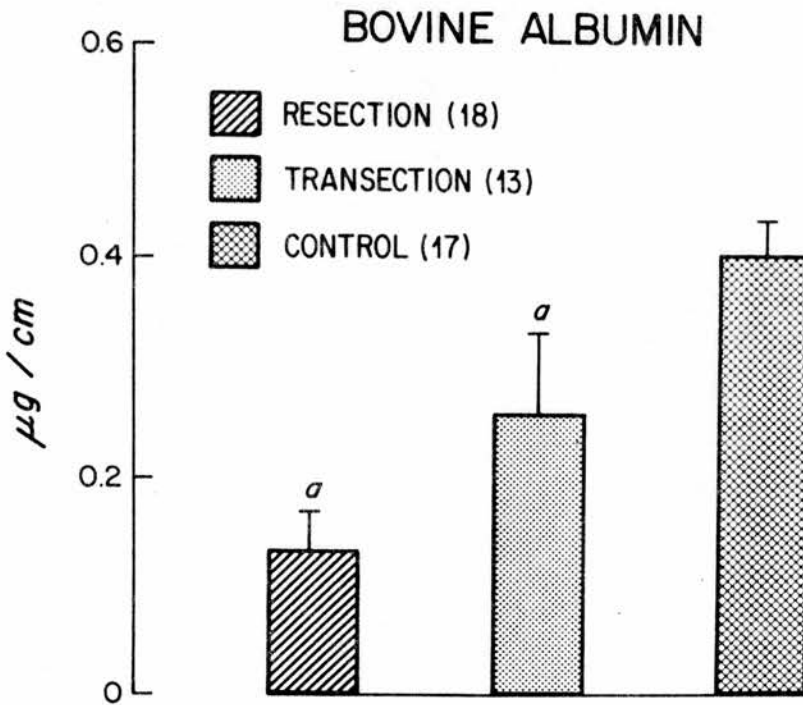


Fig. 10.3 Serum levels of BSA per ml of rat serum per cm of residual small bowel (\pm S.E.). A = $p < .05$ v controls (Wilcoxon's rank sums test).

not reduce the incidence or the degree of vacuolation in association with the changes in the other parameters in mucosal maturation.

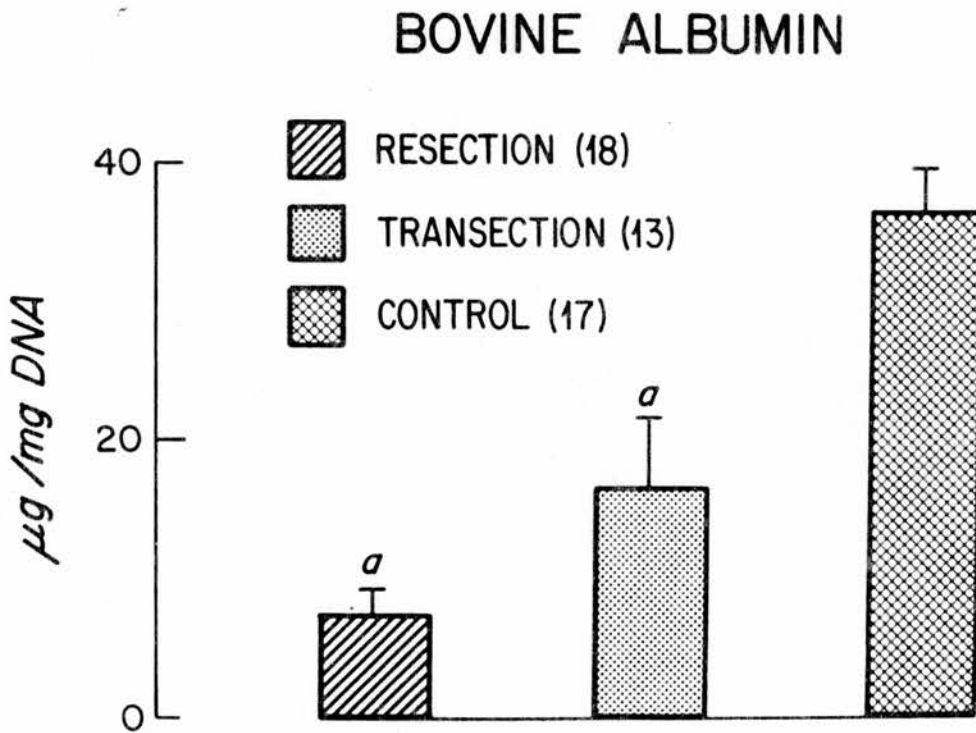


Fig. 10.4 Serum levels of BSA per ml of rat serum per mg DNA in the residual intestine (\pm S.E.). A = $p < .01$ v controls (Wilcoxon's rank sums test).

Table 10.3 Intestinal length cm (\pm S.E.)

		Proximal	Mid	Distal
Ten day old	n = 31*	5.2 ± 0.1^a	15.41 ± 0.2	5.2 ± 0.1^a
Resection	n = 18**	8.9 ± 0.3		9.7 ± 0.3^{bc}
Transection	n = 13**	8.1 ± 0.3^a	16.95 ± 0.3^d	8.3 ± 0.2^c
Control	n = 17**	8.9 ± 0.2^a	22.55 ± 0.5	8.9 ± 0.2^a

*measured *in situ* in anaesthetized rats

**measured suspended with 2 g tension after sacrifice

a = 20% of small bowel length

b = $p < .01$ v transection

c = $p < .05$ v controls

d = $p < .001$ v controls

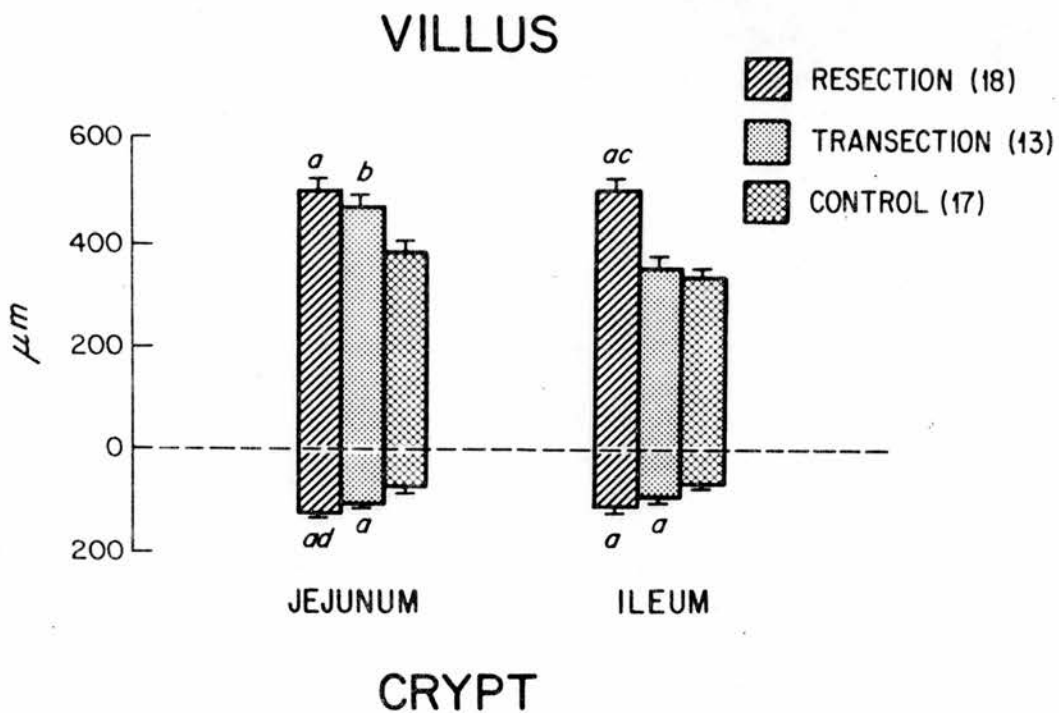


Fig. 10.5 Villus height and crypt depth (\pm S.E.) at 15 days of age. A = $p < .001$ v controls; B = $p < .01$ v controls; C = $p < .001$ v transection; D = $p < .02$ v transection.

Table 10.4 Enterocyte vacuolation

	PROXIMAL INTESTINE		
	Resection	Transection	Control
No vacuolation	4	3	4
Mild vacuolation	13	8	13
Moderate vacuolation	1	1	-
Marked vacuolation	-	1	-
Total	18	13	17

	DISTAL INTESTINE		
	Resection	Transection	Control
No vacuolation	3	1	-
Mild vacuolation	4	3	1
Moderate vacuolation	6	3	3
Marked vacuolation	5	6	13
Total	18	13	17

BIOCHEMICAL ESTIMATIONS

Cell Numbers and Cell Size

Intestinal DNA content

In comparison with control rats, the total intestinal DNA content per centimeter increased by 54% in the jejunum, and by 84% in the ileum after intestinal resection. After intestinal transection, the DNA content increased by 27% in the jejunum, by 44% in the mid-small bowel, and by 51% in the ileum (Fig. 10.6).

Intestinal RNA content

In comparison with control rats, the total intestinal RNA content per centimeter increased by 292% in the jejunum, and by 200% in the ileum after intestinal resection. After intestinal transection, the RNA content increased by 147% in the jejunum, by 118% in the mid-small bowel, and by 109% in the ileum after intestinal transection (Fig. 10.7).

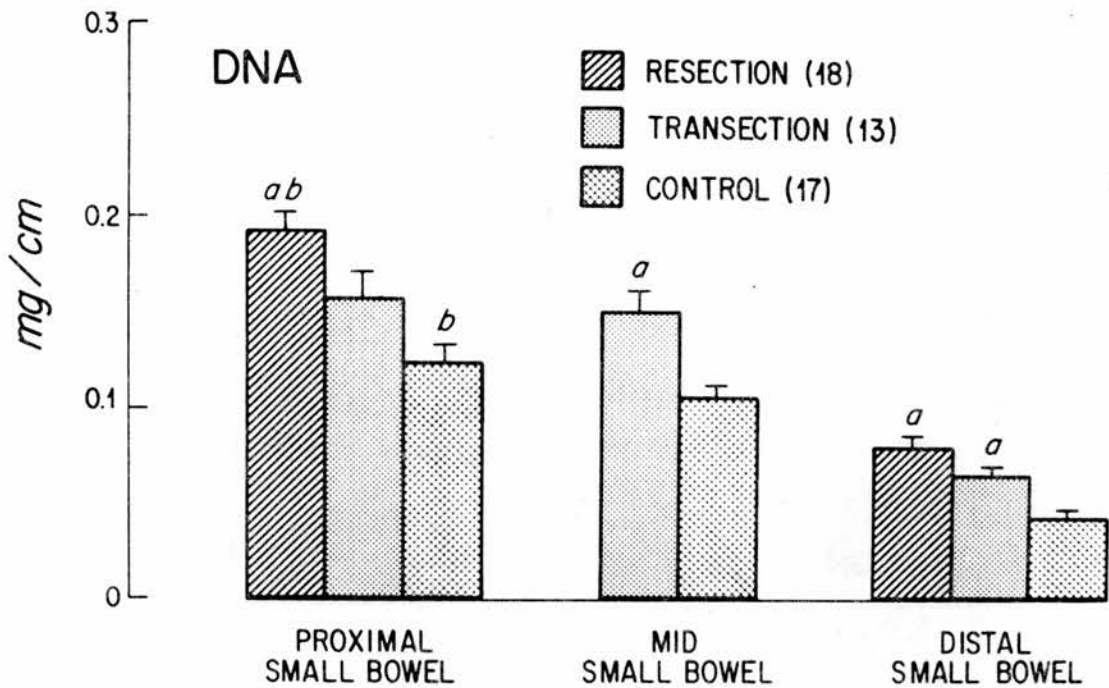


Fig. 10.6 DNA content of the residual intestine (\pm S.E.).
 A = $p < .001$ v controls; B = $p < .01$ v transection.

Intestinal RNA:DNA ratios

In comparison with control animals, the RNA:DNA ratios increased by 151% in the jejunum, and by 52% in the ileum after intestinal resection. After intestinal transection, the RNA:DNA ratios increased by 93% in the jejunum, by 62% in the mid-small-bowel, and by 39% in the ileum (Fig. 10.8).

Intestinal Enzyme Activity

Sucrase activity per centimeter

In comparison with control rats, the sucrase activity per centimeter increased by 300% in the jejunum and by 205% in the ileum after intestinal resection, and by 54% in the jejunum and by 27% in the ileum after intestinal transection (Fig. 10.9).

Sucrase activity per mg DNA

In comparison with control animals, the sucrase activity per mg DNA increased by 148% in the jejunum, and by 66% in the ileum after intestinal resection. Activity per mg DNA increased by 18% in the jejunum, but decreased by 19% in the ileum, after intestinal transection (Fig. 10.10).

Lactase activity per centimeter

In comparison with control rats, the lactase activity per centimeter increased by 190% in the jejunum and by 200% in the ileum after intestinal resection, and by 31% in the jejunum and by 85% in the ileum after intestinal transection (Fig. 10.11).

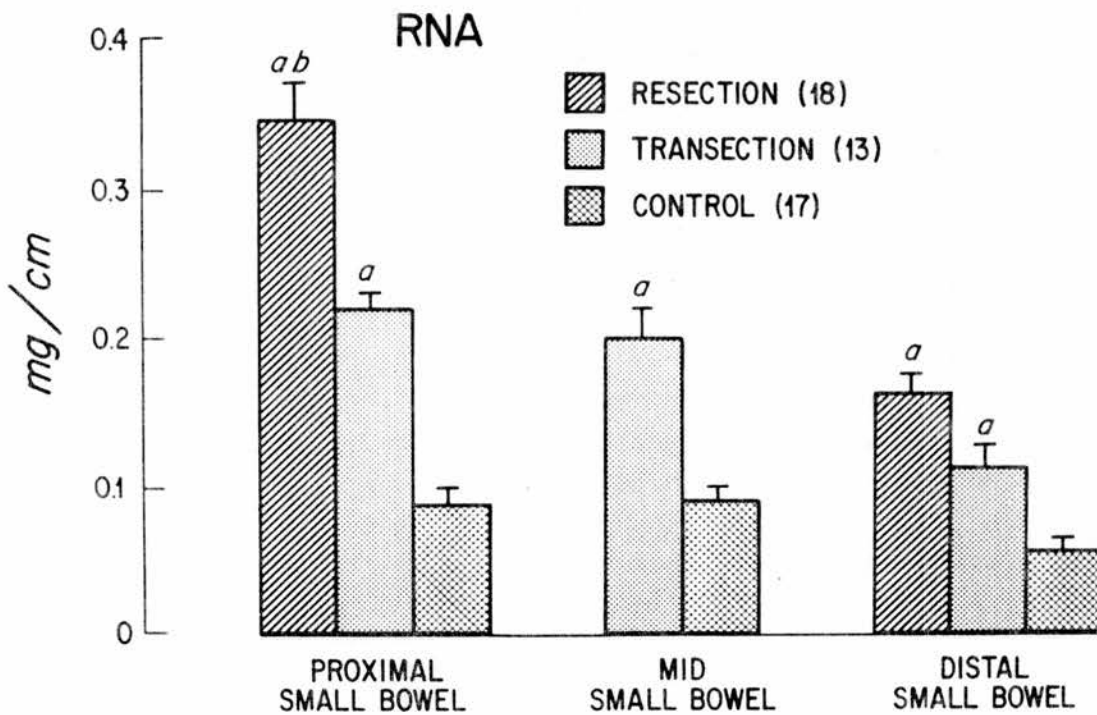


Fig. 10.7 RNA content of the residual small intestine (\pm S.E.).
A = $p < .001$ v controls; B = $p < .02$ v transection.

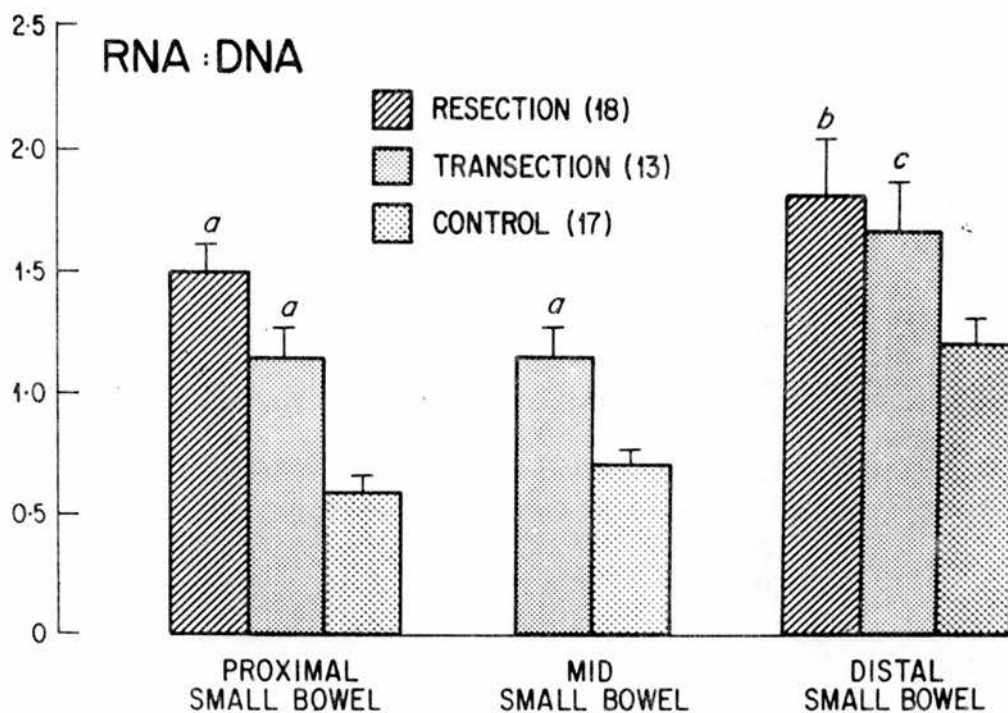


Fig. 10.8 RNA:DNA ratios in the residual small intestine (\pm S.E.).
 A = $p < .001$ v controls; B = $p < .02$ v controls; C = $p < .05$ v controls.

Lactase activity per mg DNA

In comparison with control rats, the lactase activity per mg DNA increased by 94% in the jejunum and by 70% in the ileum after intestinal resection, and decreased by 1% in the jejunum and increased by 13% in the ileum after intestinal transection (Fig. 10.12).

DISCUSSION

The trans-mucosal transport of immunologically recognisable BSA into the blood stream was sharply decreased five days after partial enterectomy in suckling rats, despite the marked mucosal hyperplasia. The effect was non-specific, as it also occurred

after intestinal transection (Fig. 10.13). The mechanism of this enhanced exclusion appeared to be accelerated mucosal maturation.

Intact antigenic macromolecules are normally transported across the intestinal mucosa and yolk sac of a variety of laboratory animals (45, 46, 47, 212, 301). But, in rabbits and rats, macromolecular transport decreases as one of the normal features of intestinal maturation (1, 132, 144, 212, 259, 357, 363, 366). Nevertheless, with sensitive radioimmunoassay techniques, intact enterically presented antigens can still be detected in the serum of mature animals (145, 276). Furthermore, in the immediate period after intestinal damage, and with the intestinal epithelial alterations associated with infectious or allergic enteropathy (31,

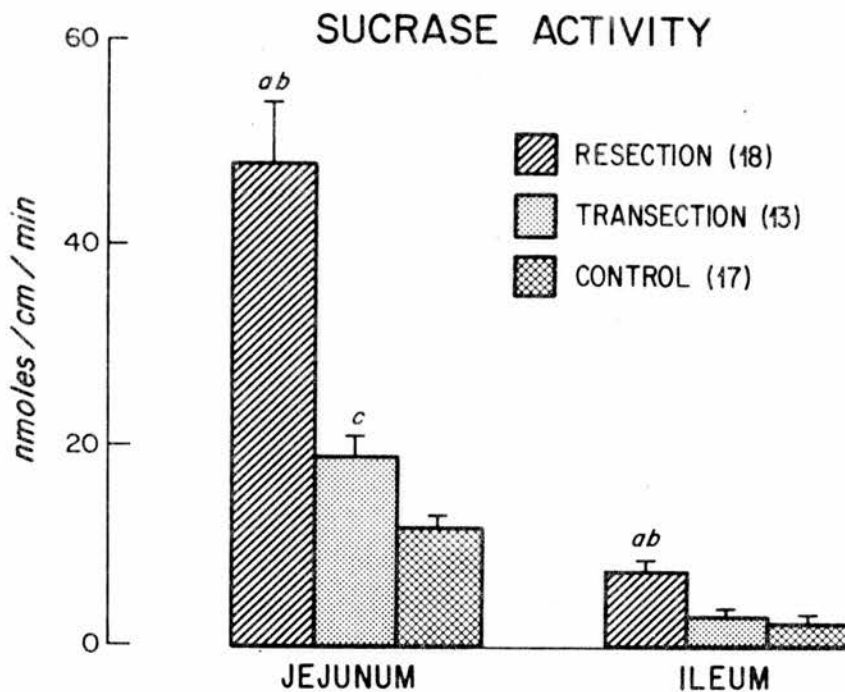


Fig. 10.9 Sucrase activity in the residual small intestine (\pm S.E.). A = $p < .001$ v controls; B = $p < .001$ v transection; C = $p < .01$ v controls.

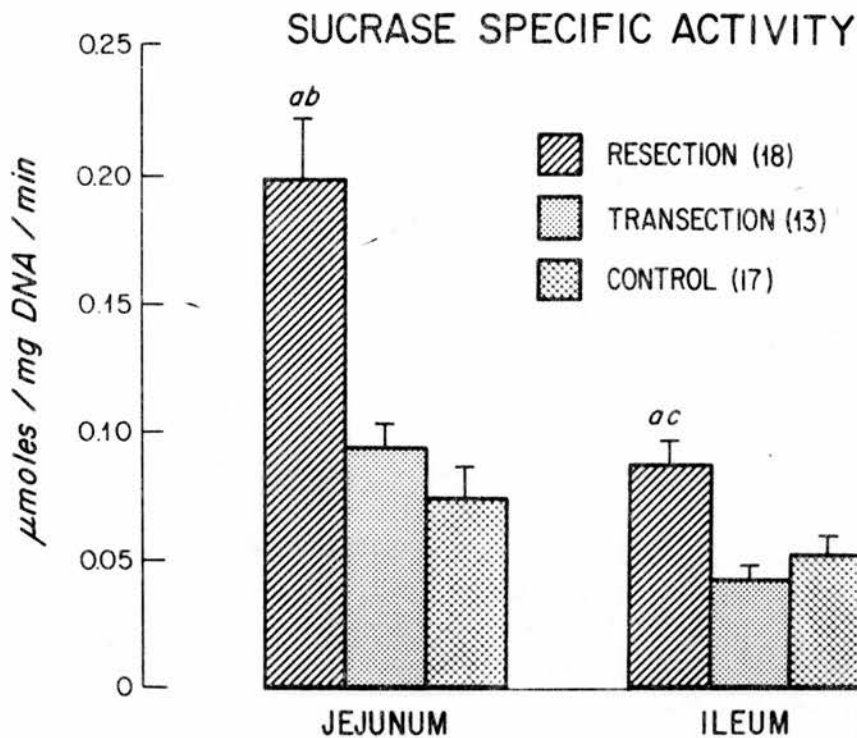


Fig. 10.10 Sucrase specific activity (\pm S.E.). A = $p < .01$ v transection; B = $p < .001$ v controls; C = $p < .02$ v controls.

300), there is increased transport of macromolecules across the mucosal barrier (31, 75, 293, 294, 343, 366, 396). Therefore, intestinal resection, with subsequent damage to the residual intestine, could theoretically be associated with loss of mucosal barrier function, and result in increased intestinal absorption of intact macromolecules, especially in immature animals, where macromolecules normally cross the intestinal epithelium. Nevertheless, this study demonstrated that the overall effect of intestinal resection was to produce a situation where there was enhanced exclusion of enterically presented antigen. Therefore, intestinal surgery appears to accelerate this particular characteristic of intestinal mucosal maturation.

Other morphological and biochemical features of mucosal maturation were also demonstrated after intestinal surgery in the suckling rat, and are similar to those that occur during the normal weaning process (255). One of the most reliable, and most used, markers of mucosal maturation is the appearance of sucrase activity (147). Sucrase activity per centimeter and per cell appeared early after intestinal resection, and to a lesser extent after intestinal transection. The increase in lactase activity per mg DNA after intestinal resection was, however, unexpected, as lactase activity normally falls while the intestinal mucosa matures (255, 320, 359). Nevertheless, stress induced mucosal maturation is not associated

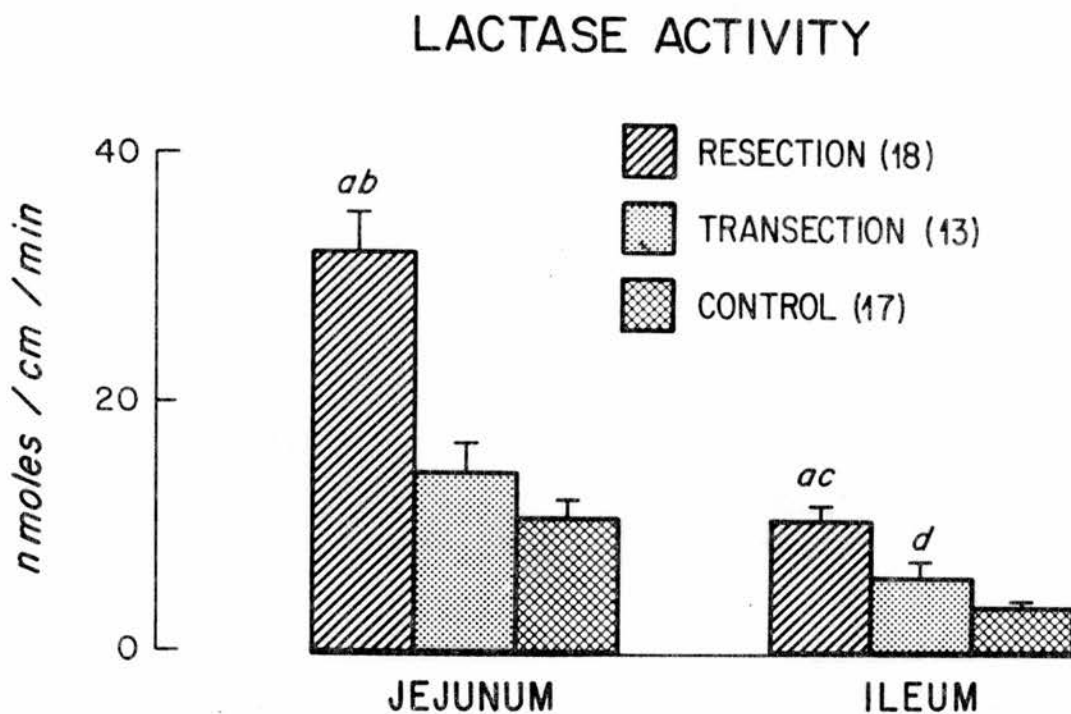


Fig. 10.11 Lactase activity in the residual intestine (\pm S.E.).
 A = $p < .001$ v controls; B = $p < .001$ v transection;
 C = $p < .01$ v transection; D = $p < .01$ v controls.

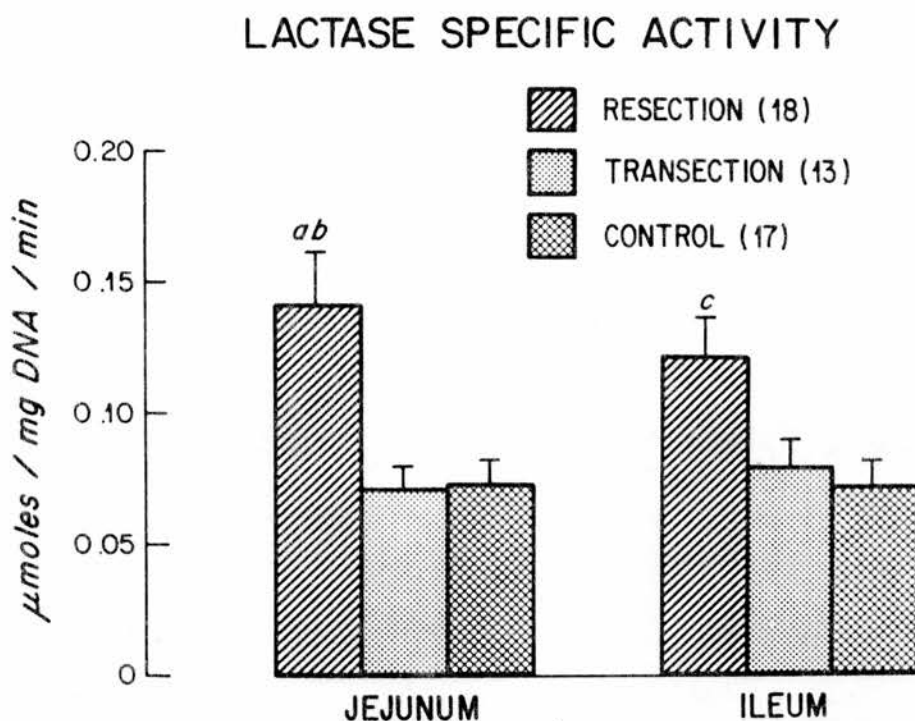


Fig. 10.12 Lactase specific activity in the residual intestine (\pm S.E.). A = $p < .02$ v transection; B = $p < .05$ v controls; C = $p < .01$ v controls.

with a precocious decrease in lactase activity to the same degree as stress induced increases in sucrase activity (211, 255, 359). Furthermore, as enterocytes migrate from the crypt to the villus tip in the suckling rat, the lactase specific activity increases (46; see Chapter 4). Therefore, as the villi increased in height after intestinal resection, the lactase activity may be expected to be greater in the cells near the tip of the taller villi rather than in the cells close to the tip of the normally smaller immature villus. Therefore, under these very limited circumstances, where the intestine is undergoing an adaptational change before the suckling period has ceased, lactase appears to be an inducible enzyme, whereas in the older rat it is not (149).

The increased stress of intestinal resection was not associated with the disappearance of supranuclear vacuoles, and the normal, more marked, supranuclear vacuolater was demonstrated in the cells of the distal intestine. Normally, these vacuoles disappear as antigen absorption decreases, at the time of normal weaning (77, 147, 156, 325), and will disappear abnormally early after injections of corticosteroids (77). Possibly, insufficient time had elapsed for the cell population to have changed in the

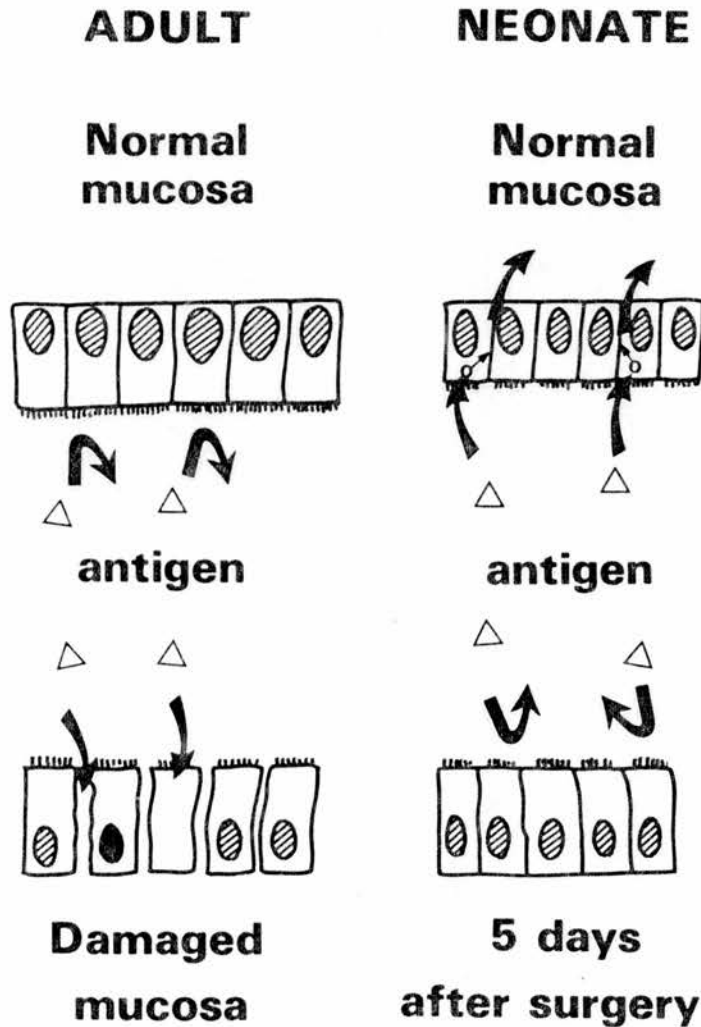


Fig. 10.13 Although intestinal damage allows antigens through the mucosal barrier in the adult animals, intestinal resection and transection accelerates mucosal maturation so that the larger more mature cells now exclude antigen in a similar manner to adult enterocytes.

five days following surgery (202) or the adrenal response to surgery was not mediated by corticosterone, but by cortisone, which does not change the enterocyte cell population to one without vacuoles but, nevertheless, can enhance antigen exclusion in the cell population that lines the villi (77). One further possibility is that the adrenal cortex is not implicated in this aspect of mucosal maturation after intestinal resection, although from the results of previous studies as to the effects of adrenalectomy and corticosteroids, this seems unlikely (43, 255).

While the structural and biochemical alterations recorded in the intestinal mucosa must represent changes in the state of the intestinal epithelium, the alterations in the serum levels of BSA could have been the result of other influences, such as an increased extracellular fluid volume, an incomplete exposure of the gavaged BSA to the absorptive surface area, or increased peptic digestion of BSA after both intestinal resection and transection. As fluid retention follows major surgical stress, the observed alterations in the serum BSA levels could merely be the result of haemodilution. Arguing against volume differences between operated and control animals are the similar weights at the time of sacrifice, and the observation that all animals were suckling *ad libitum* for five days after the surgery. Furthermore, after intestinal resection, the intestinal fluid losses that normally follow (381) would cause haemoconcentration and not haemodilution and, therefore, the greatest concentration of BSA would be expected in those animals undergoing intestinal resection, not controls.

Operated and control animals showed a similar intestinal transit time and, while intestinal peptidase were not quantitated, the loss of intestinal surface area would be expected to reduce their total activity, not to increase it. Therefore, again, BSA levels should have been increased after resection, not decreased.

Peak serum levels of BSA were found at 1.5 hours in those animals undergoing intestinal resection. Transit time to the ileo-caecal valve was approximately 2.5 hours in all animals. Therefore, to avoid measuring immunoreactive peptides absorbed from the colon (8), we elected to study the rats 2.5 hours after gavage with BSA. Since peak levels of BSA were found at 4.5 hours in control rats, the differences in serum immunoreactive BSA which we observed may have been even greater if we had carried out our definitive study at 4-5 hours after gavage.

The serum levels of BSA recorded here must represent an absorptive process that can be altered by surgery. If serum levels of BSA were simply taken at absolute values, then, obviously, serum levels would be lower in those animals that had lost 60% of their small intestine. When the serum levels were related to the length of the residual intestine, however, the differences in the absorption of BSA remained. When the serum BSA levels were related indirectly to the cell population in the residual intestine, the absorption per mg DNA was still decreased after intestinal surgery. Furthermore, in those animals that had undergone intestinal transection that had an essentially normal intestinal length, the absorption of BSA also decreased after surgery.

In these studies, we have demonstrated that intestinal resection during the suckling period of the infant rat results in several morphological and physiological changes, which resemble the normal process of mucosal maturation. These changes, however, are induced earlier than normal. Whether these changes are stress related with the attendant systemic hormonal response remains to be determined. Nevertheless, the response to intestinal resection in the suckling rat may help to explain the marked functional and structural adaptation after intestinal resection in human neonates (182, 295, 319, 360).

Acceleration of the development of the enteric mucosal barrier may contribute to the protection of the infant rat from harmful enteric antigens in the post-operative period. The increase

in the epithelial cell population as measured by increase in DNA per unit length, and the increases in enterocyte enzyme activity, may allow the infant rat with a shortened gut the opportunity to increase the digestion and absorption of nutrients, so increasing the potential for survival in the post-operative period.

CHAPTER ELEVEN

FINAL DISCUSSION AND CONCLUSIONS

These studies have demonstrated that the residual intestine of the suckling rat is capable of marked compensatory mucosal growth after major small bowel resection. This regeneration includes both cellular hyperplasia and hypertrophy. As a result, there is marked villus elongation to accommodate the increased numbers of larger cells, and there is increased crypt depth, with increased numbers of mitoses within the crypts, presumably to maintain the increased cell population. At the same time, mucosal function appears to be enhanced, with increases in both lactase and sucrase activity. Nevertheless, premature mucosal maturation appears to have a large part to play in the mucosal response to intestinal surgery in suckling rats. This maturation is, however, non-specific, as similar, but less marked, responses are seen after intestinal transection. Furthermore, this non-specific acceleration of mucosal maturation after surgical stress was sufficiently marked in the first experimental study to demonstrate that intestinal transection was not an adequate control for intestinal resection, especially where mucosal enzyme activity is concerned. Nevertheless, the same experiment demonstrated that compensatory regeneration will occur after intestinal resection in immature rats over and above the accelerated mucosal maturation associated with intestinal transection. As a result, there are increases in villus height and, to a lesser extent, intestinal DNA content, over and above those seen after intestinal transection.

STRUCTURAL CHANGES

In the breast milk and the BSA studies (Chapters Nine and Ten), where normal controls were used as well as animals undergoing intestinal transection, intestinal resection was seen to produce marked intestinal adaptation in the form of increased villus height, crypt depth, DNA content, cell size and sucrase activity, and that intestinal transection mimics these responses, but to a lesser degree. During the five day study period, however, intestinal elongation over and above normal growth was not demonstrated. Furthermore, there was no compensatory elongation of the residual intestine for up to 24 days after a 60% intestinal resection at 2, 3, or 4, weeks of age (Chapter Three). There was no compensatory elongation of the ileum four weeks after a 60% proximal small intestinal resection (Chapter Four), but there was compensatory elongation of the residual intestine 38 days after an intestinal resection carried out on 7 week old rats. Therefore, these studies would suggest that intestinal elongation is not a feature of intestinal adaptation after intestinal resection in the very immature rat, but does become a feature in older rats. Previous studies in older rats have demonstrated their ability to mount such a response, as intestinal elongation has been reported within 7 days of a 90% small bowel resection (307), and after a 70% intestinal resection (269) in the mature rat. Nevertheless, these observations of diminished, or absent, compensatory intestinal elongation are contrary to the observations in one clinical series (182), and the clinical observations that functional adaptation is more marked in the young (295, 296, 360). Possibly, the intestine of the very immature rat is elongating so rapidly that no further elongation is possible (198).

One further distinct difference seen after intestinal resection in the suckling rat was the apparent cellular hypertrophy. If the RNA:DNA ratio is a reliable indicator of the cell size, then these studies would suggest that, after intestinal resection in both the suckling and the older, but still immature, rat, cellular hypertrophy occurs synchronously with the cellular

hyperplasia. In a previous study of cell size, after intestinal resection in mature rats, it was suggested that the RNA:DNA ratio is an indirect method of indicating cell size, and that cell size decreased markedly soon after resection (376). Furthermore, changes in RNA:DNA ratios act as an early indicator of rapid changes in cell size, especially in the early growth responses to stress (393). Therefore, the changes in the RNA:DNA ratios recorded here imply that the intestine of the immature rat not only has the capacity to increase the absorptive cell population as effectively as the mature rat (hyperplasia, demonstrated by the increased DNA content), but the intestine of the immature, as well as the suckling, rat has the capacity to produce a cell population where the individual cell is bigger (hypertrophy, demonstrated by the increased RNA:DNA ratio). This response cannot simply reflect the increased RNA:DNA ratios seen as the intestine matures, as the RNA:DNA ratio increases between 18 and 21 days of age in the normal rat (201), and therefore could not be expected to change 38 days after an intestinal resection in a 7 week old rat (Chapter Eight). Therefore, this difference in response to intestinal resection in the immature rat must be real, and not merely another aspect of accelerated intestinal mucosal maturation. Furthermore, similar changes in the RNA:DNA ratios have previously been reported after intestinal resection in more mature rats (324), so that some doubt must be expressed as to the validity of the original study (376).

ENZYME CHANGES

After intestinal resection in the 14 day old rat, the lactase activity was seen to virtually disappear within 6 days of an intestinal resection (Chapter Three), yet, after intestinal resection in the 10 day old rat, lactase activity was markedly elevated within 5 days (Chapters Nine and Ten). This apparent paradox seems to be the result of a developmental change that is occurring between 15 and 20 days of age, and is further elucidated in Chapter Four. When lactase activity was studied in the short-term bypass experiment, at 16 days of age, the lactase

activity in the in-line intestine (after 30% had been bypassed from the luminal stream) was increased over and above the lactase activity in the same part of the jejunum in rats undergoing intestinal transection, *i.e.* only in the non-bypassed gut at 16 days of age (Fig. 4.5). Therefore, food must also be present in the intestinal lumen, as well as loss of absorptive surface area, for induction of increased lactase activity. At 21 days of age, however, lactase activity in the same in-line intestine, in the same experimental study, was now lower in those rats undergoing intestinal bypass than in those animals that had undergone intestinal transection alone. Therefore, the increased lactase activity seen after intestinal resection in the 10 day old rat is ephemeral, and is age dependent, as is the normal disappearance of lactase activity (147, 255). What may be more important is that this disappearance can be accelerated by the stress of gastrointestinal surgery alone. Nevertheless, before 15 days of age, lactase activity does not appear to be sensitive to the effects of this stress, and can, in fact, be induced, not only to greater activity per centimeter, but also to greater activity per cell. By 21 days of age, however, this potential is lost, and stress now induces a rapid loss of lactase activity.

This loss of lactase activity may have parallels in clinical practice in the human newborn, for gastrointestinal surgery frequently induces a drop in lactase activity (152). To the contrary, systemic stress of non-gastrointestinal origin does not induce this response, since rats that have starved from birth maintain a greater than normal intestinal lactase activity (127, 139, 277). Therefore, it is possible to speculate that the premature human neonate undergoing gastrointestinal surgery may subsequently undergo a short period when lactose absorption is enhanced, but which is later followed by a rapid disappearance of the increased lactase activity, as well as the normal decrease in lactase activity, so that a lactose intolerance appears, without apparent cause.

Sucrase activity, on the other hand, was enhanced, no matter what the age of the rat. In the earlier studies, this appeared to be accelerated mucosal maturation, especially as there would be very little sucrose present in the diet of 10-15 day old rats that would not have begun to forage for themselves (147). But, if intestinal maturation was the only factor, lactase activity should have decreased also after intestinal resection in the suckling rat, but, to the contrary, lactase activity increased. Therefore, at least some of the increase in the sucrase activity should represent an adaptational response. Furthermore, when the more mature seven week old rat was subjected to a 70% intestinal resection, and also fed on a diet that contained no sucrose, but contained 20% dextrose, the intestine also responded with an increased mucosal sucrase activity in both the jejunum and the ileum. Again, this response could not represent an accelerated mucosal maturation in a mucosa that should have already been mature, but appears to be a non-specific functional response to the high carbohydrate load (149). Nonetheless, in suckling rats, when an intestinal transection is carried out, there are also increases in the upstream jejunal sucrase activity, demonstrating that there was some element of accelerated mucosal maturation in a situation where there was no increased carbohydrate load placed on the proximal intestine. Therefore, the increases in sucrase activity after intestinal resection appear to represent a combination of accelerated mucosal maturation and a functional response to the higher carbohydrate load reaching the distal gut.

LUMINAL NUTRITION

The role of luminal nutrition is highlighted by the responses to the loss of absorptive surface area after intestinal bypass, and by the effects of intravenous nutrition after intestinal resection. In the first of the two bypass studies, despite the normal somatic growth of the suckling rat, and despite an essentially normal pattern of development in the non-bypassed segment, the bypassed segment itself showed several

distinct abnormal growth and maturation patterns, which were, to some extent, confirmed by the second of the two studies.

In bypassed intestine, villus height and crypt depth were reduced. The reduction in crypt depth was apparent within 12 days (Chapter Four), while the reduction in villus height was apparent by four weeks after ileal bypass in the suckling rat (Chapter Five). After parenteral nutrition, however, reductions in crypt depth and villus height were apparent within 10 days, despite any residual stimulus for compensatory mucosal growth that may have emanated from pancreatico-biliary secretions still present in the gastrointestinal tract. Possibly, these observations would suggest that, where food is present in the rest of the gastrointestinal tract after bypass, an enterotropic factor released from the foreshortened in-line intestine was still partly stimulating the crypts in the bypassed loop. Hence the delay in the appearance of the diminished crypt growth. Furthermore, the absence of luminal nutrition from the intestine of the suckling rat does not merely inhibit mucosal growth.

Intestine does not develop normally in the absence of luminal nutrition, despite the observations that foetal intestine grafted into an adult host will exhibit many of the normal events of mucosal maturation (105, 187, 188, 199). While these foetal studies have demonstrated changes in enzyme concentrations, they have failed to demonstrate, until recently, the effects that such a procedure might have on cell kinetics within the grafted gut. When gut is grafted in this manner, villus height is decreased in comparison to normal. Furthermore, at the time that the normal increases in crypt cell production should have occurred, at the usual time of weaning, crypt cell production did not increase in the iso-grafts (243), a similar demonstration to the findings of Chapters Four and Five. Nevertheless, this present study has, in addition, demonstrated the effect that bypass has on the normal increase in rate of cell migration that occurs at weaning, and has also demonstrated that the inhibition of villus height and crypt cell proliferation will

occur in the face of any potential neuro-humoral feed-back from the shortened in-line intestine. What is more, this form of bypass in the suckling animal has allowed for an examination of the mucosal architecture in both the normal in-line, and the bypassed, bowel within the same animals. This study, therefore, demonstrated that the absence of luminal nutrition prevented the normal rapid acceleration in cell migration that occurs at weaning, and at the same time demonstrated the association between this event and the absence of the normal changes in lactase activity. For how long these changes are inhibited remains to be determined.

The second bypass study reconfirmed that the absence of luminal nutrition for any length of time inhibits both longitudinal and mucosal growth within the small bowel (196, 258). But this study demonstrated, in addition, that the presence of food elsewhere in the intestinal tract did not overcome the lack of growth in the bypassed loop. While this lack of response might argue for the absence of any humoral factor affecting the intestine in this age of animal, the site of origin of the bypassed bowel, and the ileum's known lack of sensitivity to an enterotropic factor, must be borne in mind (see also Introduction section 6.2). Nevertheless, the lack of longitudinal growth in the bypassed ileum raises the interesting speculation that the local presence of undigested food in the distal intestine of a growing animal may well determine the eventual length of the intestine by a negative feed-back mechanism, where undigested food in the distal bowel stimulates the intestine to grow. Furthermore, as milk contains identified and unidentified growth factors in significant quantities (7, 62, 142, 194, 195, 289, 342), these growth promoting factors may amplify the feed-back mechanisms. Food in the distal intestine may stimulate the release of enteroglucagon, an effective stimulator of crypt cell proliferation (6, 32, 33, 34), and this hormone could be an effective mediator of such a negative feed-back mechanism. On the other hand, if the bowel were to respond to an enterotropic factor released by the presence of food in the proximal intestine, then the intestine would never stop growing.

In the parenteral nutrition study, the absence of luminal nutrition throughout the intestinal tract produced short-term inhibition of longitudinal growth both proximal and distal to the anastomosis. This did not appear to be merely the result of malnutrition, as rats were growing all the time. On the other hand, an elemental diet produced compensatory villus growth and increased crypt depth both proximally and distally, although the increased crypt depth was less marked than when rats were fed chow. Nevertheless, the parenteral nutrition, whether given by vein or by mouth, eventually induced a long-term improvement in somatic and longitudinal intestinal growth when compared with those animals fed rat chow, despite the initial inhibition of these parameters. These results would, therefore, suggest that a combination of oral nutrition and parenteral nutrition would be the best for the induction of optimal somatic growth (and hopefully general development), optimal intestinal elongation, and optimal mucosal adaptation after intestinal resection. The choice of oral nutrition remains somewhat of an enigma, as the elemental diet might be more easily absorbed than a complex diet such as milk, but a complex diet apparently induces more mucosal growth, and milk also contains many growth factors that could benefit intestinal regeneration (7, 62, 142, 194, 195, 289, 342). Certainly, the results of the breast milk study would support the contention that the growth factors present in breast milk may be of value. Whether they could be added to an elemental diet and remain effective remains to be determined.

GROWTH FACTORS

The growth factors in saliva and the polypeptide gastrin did not appear to be of value. This does not mean, of course, that the addition of known salivary growth factors would not be of value, only that the removal of the source in the salivary glands did not produce an inhibition of intestinal growth. As these growth factors are also produced elsewhere in the gastrointestinal tract, for

example, E.G.F. is found in the secretions of the human pancreas (155), and as the pancreas hypertrophies after intestinal resection (338), the local availability of epidermal growth factor in particular may well increase after intestinal resection, without any increase in production of E.G.F. from the salivary gland. Increasing the endogenous levels of serum gastrin was not a helpful procedure and, if anything, increased serum levels of gastrin were associated with delayed mucosal maturation and decreased DNA content in the jejunum.

The one study that showed promise of finding an effective aid to compensatory intestinal regeneration was the breast milk study. In that study, there was a 30% increase in the distal villus height over and above that seen after intestinal resection in similar animals fed commercial formula milk, and even some increase over and above those animals fed breast milk from later in the lactation period. Nevertheless, these observations may reflect nothing more than an associated accelerated mucosal maturation brought about by the earlier breast milk. Related studies have shown that colostrum will bring about an early mucosal "closure" to macromolecules, and this reflects one aspect of intestinal maturation (358; see also Chapter Ten). This possibility is supported, in part, by the increased sucrase activity that was found in the rats that were fed on the early breast milk, as opposed to the late breast milk, after the resection. Whatever the underlying mechanism of the increased villus height and the increased sucrase activity, both of these must be of benefit to intestinal absorption in the animal recovering from a massive intestinal resection.

The increased mucosal growth seen in those animals fed the earlier milks after intestinal resection over and above that seen after feeding commercial formula was not dramatic, and the difference between the effects of the earlier and the later milks was not marked. This lack of dramatic effect may reflect the falling growth factor concentrations found in breast milk within 2-5 days of giving birth (289) and, therefore, colostrum would obviously have been a better tool for evaluating the effects of the growth factors in

breast milk. For technical reasons, however, colostrum was impossible to obtain from the rat. Furthermore, when rats were fostered to dams that had been lactating for only three days, repeat fostering on a daily basis was impossible to achieve without an unacceptable mortality. By the end of the five day study period, therefore, the rats that had started on three day breast milk were now on eight day breast milk, so that any beneficial effect from the growth factors would be diluted as the growth factor concentrations fell. A more effective study would be the isolation of the factor(s) responsible for the improved mucosal growth and the increased enzyme activity, and to use that factor(s) to enhance intestinal adaptation.

ANTIGEN TRANSPORT

Whereas gamma globulins are transported by specific binding sites in the proximal intestine in suckling rats (301), non-replicating antigens (83), and even replicating antigens in the form of viruses (394), are transported to the sub-microfold "M" cells of the distal intestine in suckling rodents. When these antigens gain access to the gut associated lymphoid tissue, they induce a local T cell helper response, which in turn helps B cells to produce secretory IgA. At the same time, these antigens also induce a T cell suppressor response that suppresses the systemic production of IgG, IgM and IgE (83). Hopefully, therefore, this co-ordinated response blocks the access of antigen to the general circulation but, when small quantities of antigen repeatedly get through, the suppressor response prevents a potentially damaging systemic response to enterically presented antigens (83). In the case of replicating antigens, however, suppression of the systemic response may be detrimental to the animal, especially if there is excess antigen penetration of the intestinal epithelium, for example after intestinal damage.

After intestinal resection in the human newborn, increased susceptibility to infection has been observed (235, 377), associ-

ated with abnormalities in the systemic and local immune responses (38, 235, 285). Nevertheless, the mechanism of this susceptibility to infection may not rest purely with the abnormal systemic response, as it is possible that the resection itself so alters the local defences to enteric antigens that antigens can gain access to the systemic circulation in abnormally large quantities.

After intestinal resection, there is marked mucosal hyperplasia, especially in the ileum (381). This hyperplasia may increase the number of potential sites for the transport of antigen, especially as the greatest number of sites for such transport are in the distal intestine (394), and that is the part of the intestine that shows the greatest hyperplasia after intestinal resection (381).

The decreased transport of BSA five days after an intestinal resection does not help to elucidate the cause of the increased susceptibility to infection after enterectomy. Possibly the suggestion that distal enterectomy removes a rich source of B cells is correct (235, 377). This study, however, only looked at one time point after the intestinal resection, and antigen penetration of the mucosa immediately after the resection may have been higher than at five days. Even although hyperplasia would not occur immediately (381), mucosal damage would (294). Furthermore, after intestinal maturation in control animals, the absorption of BSA could have fallen to even lower levels than those found five days after the intestinal resection, producing a reversal of the result in Chapter Ten if the studies had been carried out at a later date.

In summary, therefore, these studies have demonstrated that major gastrointestinal surgery in suckling rats induces precocious mucosal maturation, as well as marked compensatory regeneration and functional adaptation, and despite the slower mucosal growth and slower cell turnover and production in these young animals. The maturation includes the appearance of sucrase activity within enterocytes, and the exclusion of antigens. The compensatory regeneration includes increased villus height, increased crypt depth,

marked cellular hyperplasia and cellular hypertrophy and the adaptation includes increased sucrase and lactase activity per centimeter in the suckling rat. In the weanling and weaned rat, however, the potential for increased lactase activity is lost, and lactase activity actually disappears more rapidly than in control animals.

These studies would also suggest that growth factors present in breast milk may be a valuable adjunct to the treatment of neonatal short bowel syndrome.

APPENDIX ONE

BIOCHEMICAL ESTIMATIONS

PROTEINS, DISACCHARIDASES AND NUCLEIC ACIDS

PREPARATION OF THE HOMOGENATE FROM THE CRUDE SPECIMENS

Where whole gut was used for assay in suckling and weanling rats, a measured section of jejunum, ileum, or colon, was inverted over a glass rod. The specimens were already cooled, and the mesentery already removed. The specimen was then rinsed in ice cold saline solution once more. For Chapters Three and Four, the whole intestine was stored in 5 ml of 10 mM NaPi, pH 6.0, containing 0.002% triton X-100, and frozen at -20°C for later assay. In the later experiments (Chapters Seven to Ten), the specimen was frozen after opening it longitudinally, gently rinsing it in ice cold saline, and gently blotting it dry. Where older animals were sacrificed, mucosal scrapings were used (Chapters Five and Six). To prepare these scrapings, measured segments of the intestine were removed in the usual manner, and opened longitudinally. They were rinsed in ice cold saline solution again, gently blotted dry, and then placed, mucosal side up, on a glass plate kept cool by ice placed underneath it. The mucosa was removed by gently scraping it off the muscle layers with glass slides, and then stored at -20°C for later assay.

HOMEGENATE PREPARATIONS

1. Weigh the section of the ileum, jejunum or colon.
2. Homogenize in 5 vol. 10 mM NaPi, pH 6.0, containing 0.002% triton X-100.

3. Split into three fractions:

- a) Freeze sample for protein estimation.
- b) Freeze sample for enzyme study.
- c) Freeze further sample for nucleic acid estimation.

PROTEIN ESTIMATIONS

Proteins were estimated according to the method of Lowry.*
The detail of the method used was as follows:

Reagents

Solution A: 20g Na_2CO_3
4g NaCl
0.2g NaK tartrate
 H_2O to one litre

Solution B: $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 5g per litre

Solution C: 50:1 of solution A:B

Phenol: 1:1 with H_2O

Procedure

1. Total volume to be assayed 200 μl .

Sample 10 μl of the homogenate + 190 μl of H_2O or 0.1N NaOH .

Sample 25 μl of the homogenate + 175 μl of H_2O or 0.1N NaOH .

For the protein estimations for the cell migration studies, sample from the tubes that contain the sequentially sequestered cells (see Appendix Two).

For specimens from the older animals, dilute the homogenate 1:10.

2. Add 1.0ml solution C.

3. Incubate for 10 minutes at room temperature.
4. Add 0.1ml 1:1 phenol.
5. Vortex.
6. Incubate for 60 minutes at room temperature.
7. Read the optic density at 560 λ .
8. Blank: 200 μ l of H₂O or 0.1 NaOH.
9. Standard curve: use 1.0mg per ml bovine serum albumin (BSA) mixed with 0.1n NaOH.
 - 10 μ l BSA solution + 190 μ l H₂O or 0.1N NaOH
 - 20 μ l BSA solution + 180 μ l H₂O or 0.1N NaOH
 - 30 μ l BSA solution + 170 μ l H₂O or 0.1N NaOH
 - 40 μ l BSA solution + 160 μ l H₂O or 0.1N NaOH
 - 50 μ l BSA solution + 150 μ l H₂O or 0.1N NaOH
10. Subtract the blank reading from the unknowns before calculating the protein concentrations.

DETERMINATION OF DISACCHARIDASE ACTIVITY (TSUOBOI MODIFICATION*)

1. Take homogenate of specimen and dilute with buffer (10mM NaPi, pH 6.0, 0.002% triton X-100) to desired concentration (*e.g.* 1:10 for sucrase, 1:100 for maltase, and 1:1 for lactase). The dilution finally chosen will be that which puts the final reading into the range of the standard curve.
2. The estimations were done at two concentrations, or in duplicate. For the two concentration method: pipet 50 μ l duplicate samples into test tube (A = 25 μ l unknown + 25 μ l 10mM NaPi, pH 6.0, 0.002% triton X-100 (the buffer), and B = 50 μ l of unknown).

3. Set up the blanks or controls:
 - a) Reagent blank: no enzyme (the diluted homogenate) + 50 μ l buffer + 200 μ l substrate.
 - b) Enzyme control for lactose only, as the 1:1 solution is thick enough to have a significant optical density. 25 μ l enzyme + 225 μ l buffer + no substrate.
 - c) Statzyme blank: no enzyme + 250 μ l buffer + no substrate.
4. Place tubes in a 37° C water bath.
5. Add 200 μ l substrate to each tube except the enzyme and the statzyme blank.

Substrate concentrations used were:

0.180M lactose
0.0156M maltose
0.0375M sucrose
6. Start the timer as the substrate addition is started. The time for the reaction to take place may be allowed to vary from 20-40 minutes, but the exact time must be known for the calculations.
7. When the assay time period chosen is completed (usually 30 minutes), add 250 μ l statzyme solution to each tube.
8. Incubate for a further 30 minutes at 37° C.
9. Add 500 μ l H₂O.
10. Read O.D. at 500 λ .
11. Glucose for the standard curve: use 0.001M dextrose, and put 10, 20, 30, 40, 50 μ l into tubes. Make each up to 250 μ l with buffer, but add no substrate. Add statzyme and H₂O.

12. Calculations: from the standard curve, $0.056 A = 10nM$ of glucose. Therefore, $1\mu M$ glucose is released if there is an optical density of 5.6 at 500λ and, therefore, μM glucose released per minute per ml of homogenate = $\text{absorbance} \div \text{incubation time} \div \text{sample volume} \div \text{dilution factor}$.

In Chapters Six to Ten, the procedure was modified slightly to use different pipetting volumes:

1. Vortex homogenate after thawing and dilute to 1:10.
2. Use $50\mu l$ diluted homogenate + $50\mu l H_2O = 100\mu l$.
3. Set up homogenate blank using $50\mu l$ homogenate + $150\mu l H_2O$.
4. Add $100\mu l$ substrate to the test sample. Sucrose = $0.0375M$, lactose = $0.188M$, and maltose = $0.0156M$, all three substrates in $50mM NaPi$ at pH 6.0.
5. Run 3 tubes of substrate blank using $100\mu l$ substrate + $100\mu l H_2O$.
6. Run 3 tubes of statzyme blank = $200\mu l$ of water only.
7. Incubate all at $37^\circ C$ for 30 minutes.
8. Then add $200\mu l$ statzyme to all tubes, making the volume in all tubes $400\mu l$.
9. Then incubate for another 30 minutes.
10. Then add $600\mu l H_2O$ at room temperature to make $1.0ml$, and read the O.D. at 500λ within one hour, using H_2O as a blank.

For lactose solution, add $0.2ml$ of fresh $1.0m$ *p*-chloro-mercuribenzoate (PCMB) to the substrate (PCMB and lactose solution do not store well), to inhibit non-brush border lactase activity (Asp*).

NUCLEIC ACID ESTIMATIONS

RNA Determinations

RNA estimations were carried out by the method of Scott*:

1. Precipitate 0.200ml of the homogenate in 3.0ml of 0.3N PCA. Vortex. Ice for 5 minutes. Spin at 9,000 revs. per min. for 5 minutes, remove the supernatant, and discard the supernatant (the acid soluble fraction).
2. Repeat step 1, but only add 2.0ml of the PCA to the pellet from step 1.
3. Add 4.0ml 80% ethanol to the pellet. Vortex. Ice for 10 minutes. Spin at 9.0k for 5 minutes. Discard the supernatant (the lipids).
4. Add 4.0ml cold alcohol-ether. Vortex. Let it sit at room temperature for 5 minutes. Spin at 9.0k for 5 minutes. Discard supernatant, and repeat. Two washings usually are enough to get a pellet white.
5. Evaporate the ether by inverting tubes at a slant on a paper towel for approximately 15-20 minutes.
6. Add 2.0ml 1N NaOH. Vortex. Allow to stand one hour at room temperature. Vortex occasionally.
7. Add 0.4ml 6N HCl. Vortex. Ice 2-5 minutes. Spin at 9.0k for 5 minutes. The supernatant should be clear (if not, spin in Sorvall at 9.0k for 10 minutes).
8. Remove, and collect the supernatant. Filter through glass wool in Pasteur pipet.
9. Add 1.0ml RH solution to pellet. Vortex, ice 2-5 minutes, spin for 5 minutes, collect the supernatant, and add it to the supernatant from step 8. This is the RNA fraction.

10. Dilute as required (for mouse intestine use 1.0ml of the solution and 2.0ml of water, for the young rat do the same, and for the older rat dilute 0.5ml of the solution with 4.5ml of water).
11. Read at 260 and 280 λ U.V.

DNA and DNA Specific Activity (Tritiated Thymidine Incorporation Into DNA) Estimations

For the DNA estimations, Hinrich's* modification of Burton's* method was used:

1. Precipitate an aliquot of the homogenate (200 μ l for the older rats, 500 μ l for the suckling rats and the mice) in 3.0ml of 0.3N PCA. Vortex, ice for 5 minutes, spin at 9.0k for 5 minutes, and discard the supernatant acid soluble fraction.
2. Repeat step 1 using 2.0ml of the PCA.
3. Add 2.0ml of 0.5N PCA to the pellet and vortex. Heat with the standards at 70°C for 15 minutes (the DNA is released at this stage).
4. Standard is calf thymus DNA 1.0mg per ml (in H₂O)

Vol. DNA Sol. (μ l)	H ₂ O	5N PCA
0.01ml	0.88ml	0.1ml
0.02ml	0.88ml	0.1ml
0.03ml	0.87ml	0.1ml
0.04ml	0.86ml	0.1ml
0.05ml	0.85ml	0.1ml
0.08ml	0.82ml	0.1ml
0.09ml		0.1ml

5. Chill on ice. Spin at 9.0k for 5 minutes and remove and save the supernatant, being careful not to disturb the pellet. The supernatant contains the DNA, and the pellet contains the RNA, which can be fed into the previous assay at step 5.

6. Combine 1.0ml of supernatant and 2.0ml of the colour reagent. Add 2.0ml of the colour reagent to the standards. Vortex (the colour reagent should be made fresh on each occasion):
 - 1.5g diphenylamine (dissolve in acetic acid)
 - 100ml of glacial acetic acid
 - 1.5ml concentrated H_2SO_4
 - 0.5ml 1.6% aqueous acetaldehyde
7. Wrap completely in foil and allow them to stand overnight.
8. Read at 600 λ . Read standards first.
9. Plot the standard curve, and work back from that how much DNA was present in each ml of the original homogenate, and therefore in the total specimen.

SCINTILLATION COUNTING

Count from 0.5ml of the DNA solution (the supernatant) diluted in 8.0ml of PCS (0.5ml of 0.5N PCA is used as the blank). Count 20 μ l of tritiated toluene as the tritium standard.

LABORATORY REAGENTS

1. Phosphate buffered saline (PBS)
 - KCl 0.2g
 - KH_2PO_4 0.2g
 - NaCl 8.0g
 - $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ 2.16g
 - Add H_2O to one litre.
2. PBS-EDTA with Cleland's
 - To one litre of PBS add:
 - 0.62g $\text{EDTA} \cdot 4\text{Na} \cdot 2\text{H}_2\text{O}$
 - 0.07g Cleland's

3. Perchloric acid (PCA)
Stock 70% or 12.6N
0.3M
0.5M
1.0M
4. Sodium pyrophosphate buffer (NaPi)
Stock 0.5M at pH 6.0
50mM at pH 6.0
20mM at pH 6.0 with 0.002% triton X-100
10mM at pH 6.0 with 0.002% triton X-100
5. Acetaldehyde
1.6mg per ml
6. Stock orcinol
1.35g ferric ammonia sulphate
2.0g orcinol
to 50ml H₂O
7. DPA and DPS scintillation fluid
8. Statzyme (Worthington) 500nM
12.5ml 1M tris pH 7.0
12.5ml 0.5M NaPi pH 7.0
Add one vial of statzyme powder.
9. Disaccharides: in 50mM NaPi, pH 6.0
Lactose: 0.188M
Maltose: 0.0156M
Sucrose: 0.0375M
10. RH solution
20ml 1N NaOH
4ml 6N HCl

11. Alcohol ether

3 vol. 95% ethanol:1 vol. ethyl ether

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APPENDIX TWO

The cell migration studies depend on the observation that, when everted intestine is persistently shaken at 37°C in PBS-EDTA-Clelands's solution, the cells that line the villi and the crypt, and the cells that make up the lamina propria, are sequentially sequestered from villus tip to crypt base (Fig. 4.2, page 77). In this manner, horizontal segments of the villus-crypt unit can be obtained in a sequential manner if the process is intermittently interrupted and the cells harvested from each segment and stored separately (Tsuboi*). Once the cells from the crypt have been shaken off the rest of the intestine, no more cells will come away.

After centrifugation of the cells from each segment, the protein content is estimated in each specimen. In this way, the total protein content of all of the cells in the villus-crypt unit is estimated in a cumulative process from villus tip to crypt base. Therefore, an estimate of where each segment came from can be made by knowing how much of the total protein was shaken off the mucosa before the segment in question was collected. This cumulative protein content is then used as an indirect measure of the percentage number of cells shaken off in the process (Fig. App. 2.1).

To try to determine how quickly these cells are migrating from the crypt to the villus tip, the animals are injected with tritiated thymidine at known time intervals before the animals are sacrificed. In this way, the radioactivity produced by thymidine can be determined in each segment of the villus-crypt unit by combining the "shake down" procedure with the injection of tritiated thymidine.

The activity of the thymidine per mg protein is determined in all of the segments shaken off the intestine. The activity in the segment with the greatest activity per mg protein is given a relative specific activity of 1.0, and all the other segments are given an activity that is a fraction of that (Fig. App. 2.1). In this

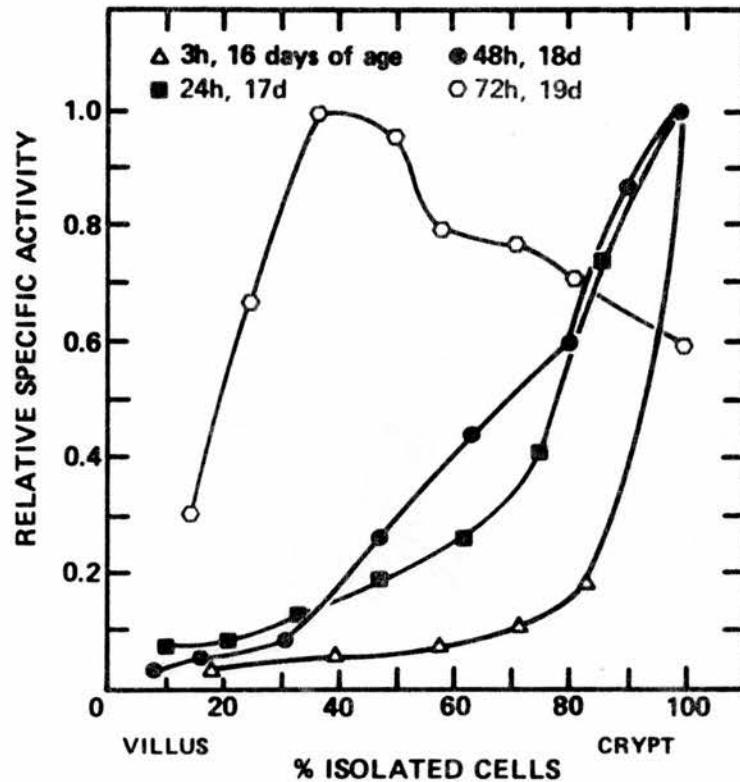


Fig. App. 2.1 Relative specific activity of the cells separated from villus tip to crypt. The segment with the greatest radioactivity per mg protein is designated to have a relative specific activity of 1.0. All other segments are given a relative specific activity proportional to this. In this way, the wave of labelled cells emerging from the crypt can be identified. Note that, when suckling rats are injected up to 48 hours before sacrifice, the major activity is still in the crypt (the righthand side of the figure), although a few labelled cells are 40% of the distance from villus tip to crypt base. Only the rat that has been injected 72 hours before sacrifice has thymidine labelled enterocytes close to the villus tip (lefthand side of the figure).

way, a curve can be constructed which will show a peak of activity which corresponds to the advancing wave of tritium labelled enterocytes at the time of sacrifice. Depending on the time interval from the time of injection to the time of sacrifice, that wave will have travelled different distances out of the crypt (Fig. App. 2.1).

Not only does the time interval between the time of injection and the time of sacrifice determine the distance travelled by the advancing wave of labelled enterocytes, but the age of the animal is also important, as the rate of cell migration changes during the weaning process (see Chapter Four and the Literature Review). In the adult rat, autoradiographic studies have shown that enterocytes will travel from the crypt to the villus tip in 48 hours. The same studies have demonstrated that enterocytes have hardly left the crypt within 48 hours of labelling in the suckling rat (Fig. App. 2.2).

While an absolute cell turnover time was not calculated in these studies, this technique can be used to do so. If injections of thymidine are given to the same age animal, under the same experimental conditions, but at differing time intervals before sacrifice, then different curves for the relative specific activity can be plotted for those time intervals (Fig. App. 2.3). As the peak activity is behind the advancing wave front of labelled cells, the point where cells have a relative specific activity of 0.5 is taken to determine the average distance travelled by labelled cells in that time. If, then, the three time points, and the distances travelled by the cells which have a relative specific activity of 0.5, are plotted against the percentage isolated cells, a straight line results, which crosses the horizontal at the time it would take for the same segment to reach the villus tip (Fig. App. 2.3, righthand side).

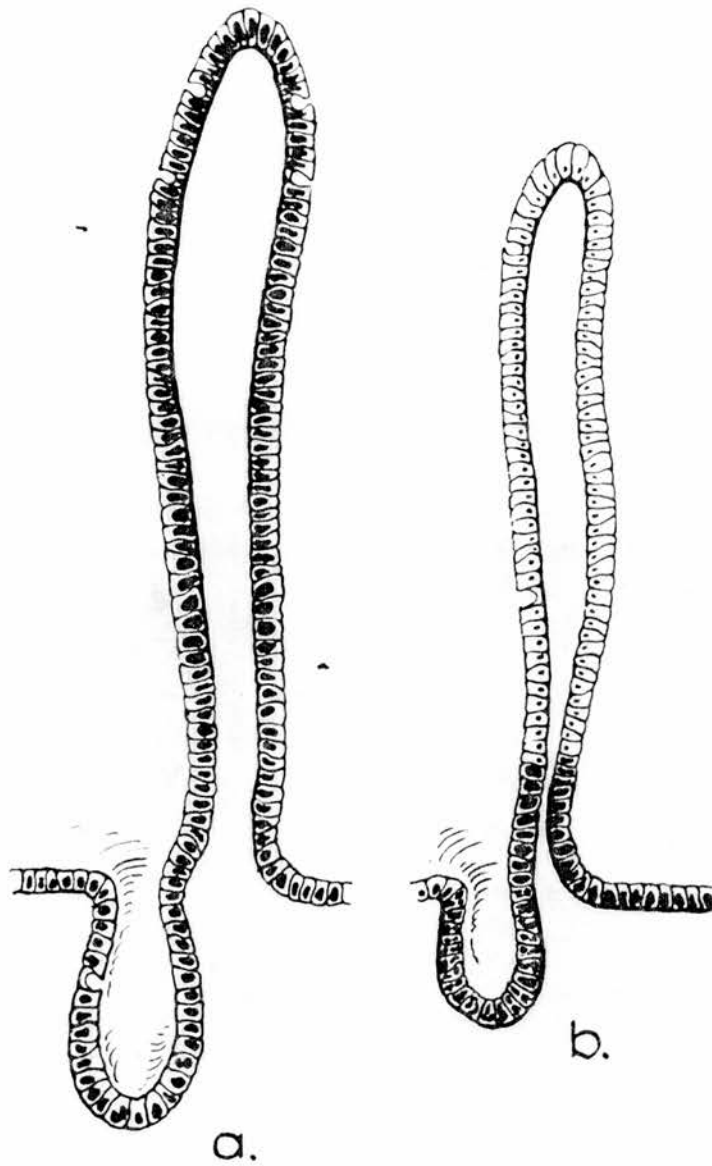


Fig. App. 2.2 Diagrammatic representation of the distance travelled by enterocytes 48 hours after the intraperitoneal injection of tritiated thymidine into adult and suckling rats (Koldovsky*). On the lefthand side, the adult rat has taller villi with labelled cells at the villus tip, whereas on the righthand side, the suckling rat has labelled cells only just emerging from the crypt, 48 hours after the injection of thymidine.

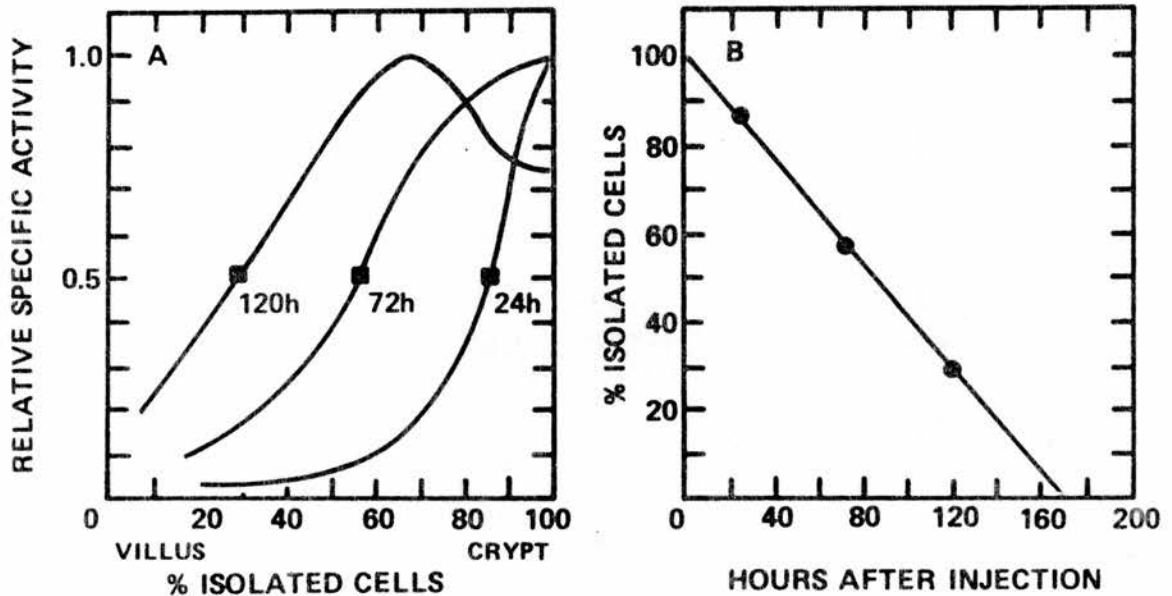


Fig. App. 2.3 Calculating the cell turnover time from the "shake down technique". Tritiated thymidine has been injected into suckling rats at three time intervals, and the resultant curves of relative specific activity plotted against the percent of cells isolated from villus tip to crypt. The "average" distance travelled by labelled cells after the injection of thymidine is taken as that point at which cells have a relative specific activity of 0.5 (lefthand figure). These times are then plotted against the percent cells isolated. The line constructed crosses the horizontal (the villus tip) at the time it should take for these cells to reach the villus tip. These observations have been checked against conventional autoradiography.

THE PROCEDURE

Preparation of Cell Migration Studies

1. Take a measured segment of ileum or jejunum.
2. Invert over a rod.
3. Rinse in ice cold normal saline solution.
4. Place in 5ml PBS-EDTA-Cleland's solution.
5. Place in 37°C bath, and shake vigorously.
6. When the solution is thoroughly cloudy (usually this takes about 5 minutes for each segment), remove the intestine from that flask, and replace it in another flask containing 5ml PBS-EDTA-Cleland's.
7. Take the original PBS-EDTA-Cleland's with its contained cells, centrifuge at 2,000 revs. per min. for 10 minutes. Discard the supernatant. Freeze the pellet.
8. Repeat Steps 6 and 7 until no more cells come away from the intestine. This usually requires 6-10 repeats. Examine the residual intestine of random specimens to check that all of the crypts and villi are removed.
9. Estimate the protein content, and carry out scintillation counting on the pellets at a convenient time.
 - a) Add to the pellet 20mM NaPi at pH 6.0 to 1.0ml.
 - b) Sonicate the pellet.
 - c) Add 500 μ l to scintillation vial, and add 50 μ l of 1.0N NaOH (take 10 μ l and 25 μ l of the suspension after sonication for the Lowry protein estimation).
 - b) Incubate at 37°C for 60 minutes.

- e) Add 5.0ml of ACS.
- f) Vortex, and wipe vial clean.
- g) Count for 5 minutes.

The relative specific activity is calculated by determining the maximum number of counts per mg protein obtained from any one specimen for that "shake down" procedure. That value is then expressed as a relative specific activity of 1.0, and all the other values are expressed as a decimal fraction of that.

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ACKNOWLEDGEMENTS

I wish to thank Drs. P. Sunshine, K.K. Tsuboi, L.K. Kwong and S.J. Shochat for their constructive criticism and advice in planning and executing the first two studies in this series. I especially wish to thank Drs. K.K. Tsuboi and P. Sunshine for the generous use of their laboratory space and facilities at Stanford University Medical Centre.

I also wish to thank Drs. R.A. Malt, J.E. de Vries, R.U. Boelhower, W.W.K. King and A.K.C. Li for their constructive criticism and advice in the planning and execution of studies 5-10. I would especially like to thank Dr. R.A. Malt for the generous use of his laboratory and facilities at Harvard Medical School.

I wish to thank Dr. R.E. Kleinman at Harvard Medical School for carrying out the immunoelectrophoresis to determine the serum concentrations of bovine serum albumin, and I also wish to thank Dr. J.E.A. Oscarson at Lund University for carrying out the serum gastrin assays.

Finally, I would like to thank Mr. A.N. Smith at Edinburgh University and Dr. G.P. Davidson at the Adelaide Children's Hospital for their constructive criticism in the preparation of this manuscript.

OBLIGATORY AND COMPENSATORY INTESTINAL GROWTH IN THE SUCKLING RAT

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IN NEWBORN PIGLETS, suckling produces a 22% increase in length of the small bowel vs that of nonsuckled pigs (1), and a high fat diet produces a 13% increase in small intestinal length in rats (2). We undertook a systematic study of the effects of food on ileal growth in the neonate.

MATERIALS AND METHODS

Ten-day-old male Wistar rats underwent one of three operations: resection of the proximal 60% of the small bowel, bypass of the distal 40% of the small bowel by connecting the proximal gut to the cecum, or transection at 60% of the small bowel length. End-to-end anastomoses were made with interrupted 7-0 silk, with antibiotic coverage. The rats were returned to their litters to suckle until natural weaning occurred, and were killed four weeks after operation. The length of the excised gut was measured after suspension with 5 gm mass. Mucosal scrapings and specimens for histologic examination were taken from the proximal half of the bypassed ileum and from corresponding places in rats with transected or resected gut.

RESULTS AND DISCUSSION

Results are summarized in Table 1. Unlike the ileum in adult rats (3), the ileum of the infant rat increased in length after it was excluded from the intestinal stream, thus exhibiting some obligatory growth. In the ileum distal to a transection, in which chyme of a normal composition is present, further longitudinal growth was observed. In contrast to profound increases in length in adult rats (3), however, growing ileum of the infant rats was not capable of compensatory (or adaptive) longitudinal growth after proximal resection, even though chyme with a high content of nutrients and pancreaticobiliary secretions reaches the ileum in both cases.

The contents of DNA and RNA in the mucosal scrapings demonstrated a different response. Amounts of DNA and RNA were greatest after proximal small bowel resection, less after transection, and least after

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Table 1

Procedure	Weight (gm)	N	Additional ileal length (%)	Gut circumference (mm)	DNA ($\mu\text{g}/5\text{ cm}$)	RNA ($\mu\text{g}/5\text{ cm}$)
Resection	116 \pm 1.8	14	281 \pm 16*	10.2 \pm 0.5*	1418 \pm 70*	489 \pm 69*
Bypass	117 \pm 1.2	18	191 \pm 11†	3.9 \pm 0.2†	391 \pm 24†	215 \pm 8†
Transection	118 \pm 2.2	13	265 \pm 15	8.3 \pm 0.5§	1100 \pm 61†	412 \pm 37

*Resection vs bypass, $P < 0.001$; †Bypass vs transection, $P < 0.001$; ‡Transection vs resection, $P < 0.001$; §Transection vs resection, $P < 0.02$.

exclusion of the ileum. The response of the villi and crypts was similar histologically. The circumference of the ileum follows this pattern macroscopically, but to a lesser degree.

Longitudinal growth that occurs after massive small bowel resection in the neonate is probably no more than normal growth for the period studied (4). Early oral feeding may be necessary to facilitate optimal longitudinal growth after small bowel resection in the neonate.

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Delayed Ontogenic Development in the Bypassed Ileum of the Infant Rat

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Ontogenic development continues after birth in mammalian enteric epithelium as an adaptive mechanism to extrauterine life. In the rat, particularly significant developmental changes in enteric structure, function, and cytokinetic properties occur over a short critical period (usually between 16 and 20 days of age), preparatory to dietary change with weaning. Surgical bypass of ileal segments was performed on suckling rats of 12-14 days of age, and the effect on subsequent intestinal development was studied in both the bypassed and the shortened segment remaining in continuity. The bypassed segment, although achieving normal maturational patterns of active sucrase appearance and maltase accumulation, continued to maintain coincident immature patterns of high lactase activity and low cell turnover times. The intestine in continuity showed precocious appearance of active sucrase and accumulation along with maltase to greater than control levels, accompanied by a normal coincident decline in lactase activity and enterocyte life-span. Involvement of intraluminal influences on various parameters of enteric ontogenic development is thus indicated with the effects expressed by a delay in the excluded (bypassed) segment and by stimulation in the shortened segment in continuity. Data are presented in further support of the hypothesis that the life-span of the enterocyte serves postnatally as a primary determinant of enteric lactase levels.

Ontogenic development continues in the intestine of the infant mammal as an adaptation of postnatal life. Particularly significant developmental changes occur in enteric (small intestine) epithelium accom-

panying weaning. Abrupt and dramatic changes in cell structure (1), function (2), and kinetic properties occur at this time. The functional adaptations of the rat enterocyte to dietary change with weaning include a coincident marked decrease in lactase, appearance of sucrase and isomaltase, and a rapid increase in maltase activities (2,3). The accompanying cell kinetic changes include increases in their proliferation, migration, and turnover rates (4,5), resulting in a decline in enterocyte life-span from 7-10 days in the preweaned to 2-3 days in the postweaned rat.

Enteric developmental adaptations, occurring at the time of weaning, would appear to be programmed intrinsically (6-8) and to be regulated by extrinsic humoral [including cortisone (6,9,10), thyroxine (11), perhaps gastrin (12)] and dietary-luminal factors (10,13,14). The physiologic mechanisms relating to these complex interactions remain to be established.

Experimental bypass of segments of small intestine, leaving nerve and blood supply intact, indicates involvement of strong intraluminal influences on epithelial cell proliferation, migration, and maturation sequences in the mature adult rat (15,16). On this basis, we examined the effect of intraluminal factors on ontogenic developmental changes which normally occur in the small intestine of the young rat at the time of weaning. The experimental approach involved the development of an ileal bypass in the 12-14-day-old suckling infant rat (by W. D. A. Ford) as a study model. The effect of the bypass on subsequent ontogenic developmental changes in (a) jejunal and (b) ileal segments maintained in continuity (as a shortened gut) and in the (c) bypassed ileal segment particularly was compared with normal developmental changes occurring in comparable intestinal segments from transected, littermate controls of similar age. Developmental changes involving dissaccharidase patterns and epithelial cell kinetics, which normally occur as dra-

Received June 30, 1980. Accepted December 30, 1980.

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This work was supported in part by USPHS Grant RR-00081.

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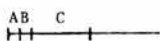
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EXPERIMENTAL INTESTINAL SEGMENTS

SEGMENT IN CONTINUITY



BYPASSED SEGMENT



CONTROL INTESTINAL SEGMENT



Figure 1. Diagram of sampling sections from experimental and control intestinal segments. Sample sections (A) of 1 cm length were taken for disaccharidase and protein measurements. Sample sections (B) of 1 cm length were taken for histologic measurements. Sample sections (C) of 4-5 cm length were taken for cell fractionations.

matic changes over a relatively short critical period accompanying weaning, were examined specifically in this study.

Materials and Methods

Materials

Wistar rats were bred and reared in our laboratory. Statzyme glucose 500 nm (Worthington), [methyl- ^3H]-thymidine at 40-60 Ci/mmol (Amersham), and disaccharides (Sigma Chemical Co. St. Louis, Mo., or Calbiochem, San Diego, Calif.) were purchased from the indicated sources.

Intestinal Bypass

Male rats 12-14 days of age were used for surgical bypass with littermates serving as controls. Animals were anesthetized with ether and injected subcutaneously with 0.5 mg kanamycin and then with a solution (50 ml/kg body weight) containing 0.9% sodium chloride, 5% dextrose, and 500 IU penicillin.

With the aid of a dissecting microscope, a midline incision was made and the distal 30% of the small intestine measured and the gut ligated between vascular arcades. The appendix was then resected and the loop of gut to be bypassed was flushed with the penicillin solution (see above) and closed with a single 6-0 silk ligature. The gut immediately proximal to the ligature was anastomosed to the cecum end to end at the opened appendix base, using a single layer interrupted 7-0 Prolene cardiovascular suture. In this design, the ileocecal valve is retained in its normal anatomical arrangement, minimizing reflux of nutrients into the loop. The contents of the gut in continuity (re-

maining proximal intestine) would thus be delivered directly to the cecum, bypassing the terminal ileum and the ileocecal valve. Control littermates were subjected to transection and reanastomosis in a similar manner.

The abdominal wall was closed as a single layer with 6-0 silk. The mortality of the procedure was approximately 20%. The animals were retained with their mothers until killed.

Tissue Sampling

At death, the entire small intestine remaining in continuity (from ligament of Treitz to the anastomosis) and the bypassed ileal segments were removed from the experimental animals. The entire small intestine (from ligament of Treitz to the cecum) was removed from the control animals. The intestinal segments were flushed with cold saline, and specified sections were taken for analyses as detailed in Figure 1.

Cell Fractionations

Intestinal segments were everted and the epithelial cells were fractionated sequentially from villus tip to inner crypt by a modification (17) of the method developed by Weiser (18). The everted segments were shaken gently in medium at 37°C containing 1.5 mM EDTA and 0.5 mM dithiothreitol in phosphate-buffered saline at pH 7.0, resulting in a sequential release of cells from villus tip to inner crypts (18). Cells were collected in successive fractions for various assays including cell protein, disaccharidases and radioactivity (see below). The amount of cells (i.e., protein) collected in the successive fractions from villus to crypt has been expressed as cumulative percents of the total cell protein released (e.g., see Figure 2).

Cell Migration

Control and experimental rats were injected with 25 μCi [^3H]thymidine intraperitoneally to provide a stable

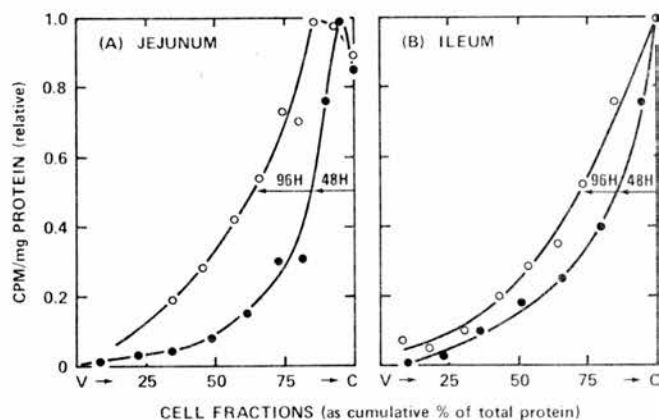
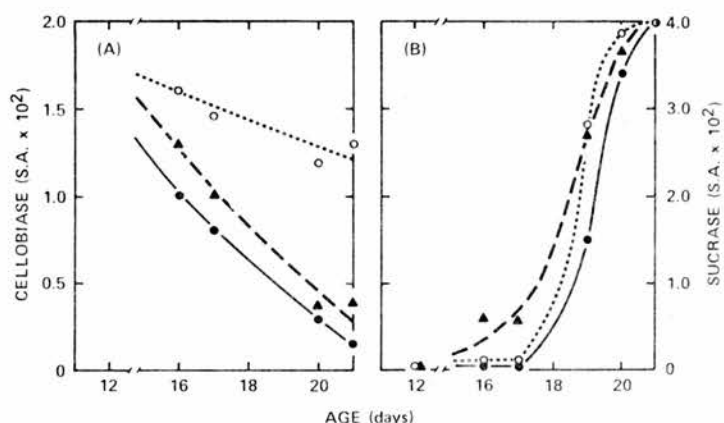


Figure 2. Distribution of radioactivity in cell fractions isolated sequentially from villus tip to inner crypt from A. jejunal and B. ileal segments of 15-day-old rats, at 48 and 96 h after a single injection of [^3H]thymidine.

Figure 3. Developmental patterns of A. lactase (cellobiase) and B. sucrase in the bypassed ileum of the suckling rat. Surgical bypasses were performed on 12-day-old rats and specified segments (see Methods) of bypassed (\circ --- \circ) and in-line (\blacktriangle --- \blacktriangle) ileal segments were assayed for enzymes subsequently at the ages indicated. Comparable ileal segments (\bullet --- \bullet) from littermate control were similarly assayed.



marker of newly formed cells originating in the crypt zone. Animals were killed at subsequent specified periods, and migration rate of labeled cells along the crypt to villus axis was determined as follows. Intestinal cells were fractionated sequentially from villus tip to inner crypt (see previous description) and distribution of labeled cells determined by radioactivity measurements in a Beckman LS-223 beta counter at 30% efficiency.

The radioactivity present in the sequentially isolated cell fractions was determined, and the relative specific activities (i.e., relative to the cell fraction of highest specific activity) were plotted in relation to the position of the cell fractions along the villus to crypt unit as illustrated in the model experiment shown in Figure 2. Distribution patterns are shown of radioactivity in sequential cell fractions prepared from jejunal and ileal segments of 15-day-old infant rats injected 48 and 96 h earlier with [^3H]thymidine. The average migration distance of the labeled cells from crypt to villus is shown at the point of the directional arrows where the relative specific activity is equal to 0.5. The migration distances are generally proportional with time, as found in our earlier studies using radioautographic methods (10), and indicate cell turnover times in excess of 10 days in the suckling preweaned animal. Cell turnover times in jejunum and ileum increase rapidly at the normal time of weaning (10) to less than 3 days when determined by these methods (see Results).

Other Measurements

Tissues were fixed in 10% formalin, embedded in paraffin, sectioned along their longitudinal axes and stained with hematoxylin and eosin. Villus height, crypt depth, and relative mitotic index (mitotic figures per half crypt, see Table 1) were determined under blind conditions by one observer (T. Colby).

Enzymes were assayed (also see references 19 and 20) routinely at 37°C in 250 μl reaction volume in 0.05 M sodium phosphate buffer pH 6.0, 0.002% Triton X-100, 0.03 M sucrose (sucrase activity), 0.015 M maltose (maltase activity), and 0.015 M cellobiose (a selective substrate for lactase). Enzyme units were expressed in μmoles substrate hydrolyzed per minute. Enzyme specific activities refer to

units of enzyme per milligram protein. Protein was estimated by the method of Lowry et al. (21).

Results

Bypass Preparation

The ileal bypass developed for the present study was designed in the terminal ileum to retain the ileal-cecal valve, preventing reflux of materials into the isoperistaltic blind loop. A marked diminution in diameter of the bypassed ileum was evident, compared to the proximal ileum in continuity anastomosed to the cecum. In this type of anastomosis colonic bacteria may reflux into the proximal segment (22) and cause malabsorption. However, since no significant differences in weights between control and experimental groups of animals were found, this did not appear to be a serious problem.

Longitudinal sections of control and bypassed distal ileal segments showed little difference by light microscopy in villus appearance. Although significant differences were not found in villus height, shallower crypts containing fewer cells in mitosis were present in the bypassed ileal segment as shown in the data summarized in Table 1.

Developmental Patterns of Lactase and Sucrase in the Bypassed Ileum

The effect of exclusion of intraluminal factors on developmental adaptations of enteric lactase decline and sucrase appearance, which occur normally at the time of weaning the young rat, were examined using the bypassed ileum preparation as a study model. Ileal bypasses were performed on infant rats at 12 days of age and subsequent changes in lactase and sucrase measured through the period of normal weaning. As shown in Figure 3A, high lactase levels

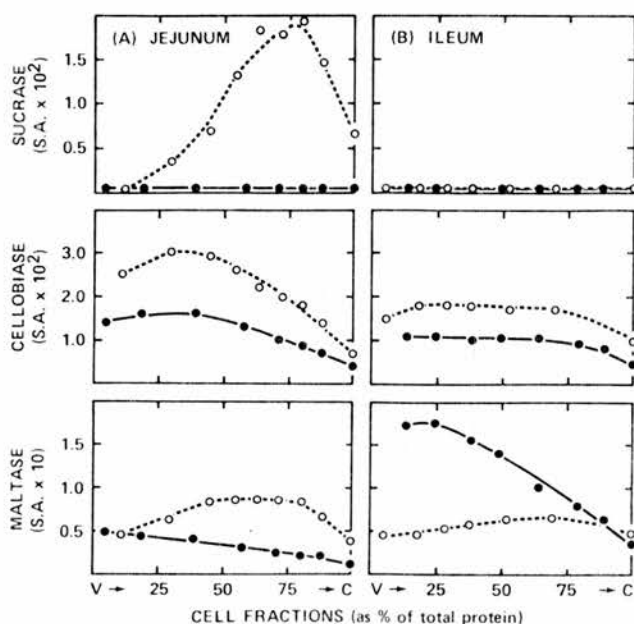


Figure 4. Patterns of disaccharidase distribution across the crypt to villus unit in A. jejunal and B. ileal segments of 16-day-old experimental and control suckling rats. An ileal bypass was performed on the experimental animal at 12 days of age. Specified segments (see Methods) of jejunum and bypassed ileum from the experimental animal (O---O) and comparable jejunal and ileal segments from the control animals (●---●) were fractionated to obtain sequential cell fractions from villus (V) to crypt (C). The isolated cell fractions were assayed for disaccharidase and protein content.

(measured as cellobiase activity) continued to be maintained in the bypassed ileum, in contrast to the normal rapid decline of this enzyme occurring in both proximal ileal segments in continuity from the experimental group of animals and ileal segments from their control littermates.

Sucrase, on the other hand, showed (see Figure 3B) generally parallel appearance in both bypassed and control ileal segments. The experimental, proximal ileal segment in continuity showed a precocious appearance of sucrase several days before its measurable presence in the bypassed and control segments.

Dissaccharidase Distribution Patterns Across the Crypt to Villus Cell Gradient

Distribution patterns of lactase, sucrase, and maltase across the crypt to villus cell gradient from experimental bypassed and control ileal segments were compared. Because the surgical bypass employed results in a significant shortening of the small intestine remaining in continuity, the adaptive effects of the shortening on disaccharidase development and distribution patterns were also examined

in proximal jejunal segments from control and experimental animals as a comparative study.

Distribution patterns of sucrase, lactase (measured as cellobiase), and maltase in enteric cells fractionated sequentially from villus tip to lower crypt are shown in Figures 4 and 5. Comparative enzyme distribution patterns between control and experimental jejunal segments are shown in the left panels and between control and bypassed ileal segments in the right panels. Patterns are obtained on animals at 16 (Figure 4) and 21 days of age (Figure 5), in which bypasses were performed in the experimental group at 12 days of age.

Animals at 16 days of age normally show preweaned enteric disaccharidase patterns of little sucrase, relatively high lactase, and low maltase activities, which is reflected by the distribution patterns of these enzymes in cell fractions from control jejunal and ileal segments (see Figure 4). Variations in the distribution of these enzymes from the control patterns are evident in both the experimental jejunal segment in continuity and the bypassed ileal segment. The jejunal segment from the shortened gut maintained in continuity shows precocious induction of active sucrase, associated at this time with only the younger cells occupying the lower villi (e.g., see reference 23), and apparent stimulated increases in both lactase and maltase activities. The bypassed ileal segment, on the other hand, shows an absence of active sucrase and maintenance of higher lactase

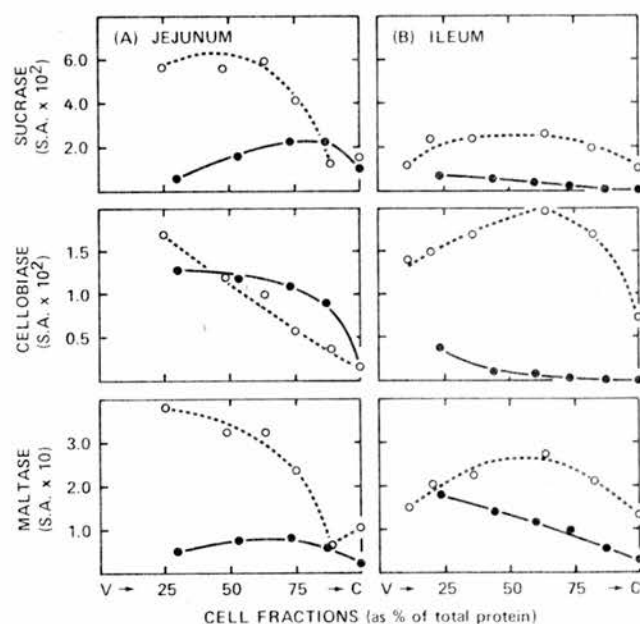


Figure 5. Patterns of disaccharidase distribution across the crypt to villus unit in A. jejunal and B. ileal segments of 21-day-old experimental and control rats. An ileal bypass was performed on the experimental animal at 12 days of age. For experimental details, consult legend to Figure 4.

Table 1. Measurements of Villus Height, Crypt Depth and Mitotic Cells in Bypassed and Control Ileal Segments in the Suckled Rat

Measurements	Bypassed ileum ^a (n = 8)	Control (Transected) ileum ^a (n = 6)	Statistical significance (p)
Villus height (mm) ^b	0.26 ± 0.20	0.31 ± 0.03	NS
Crypt depth (mm) ^b	0.29 ± 0.01	0.12 ± 0.01	<0.05
Mitoses ^c	0.39 ± 0.08	0.78 ± 0.17	<0.05

^a Rats were subjected to experimental bypass and transection (controls) at 14 days of age and intestinal segments removed for analyses 6 days later. Experimental and control animal weights did not differ significantly at the time of death. ^b Measurements were performed on the 10 largest villus-crypt units and the average value ± SEM recorded. ^c Average number of mitotic cells per half crypt (total of 10 half-crypt units examined). A "half crypt" was defined as that portion of the crypt continuous with the villus and extending from the crypt opening to the midline of the crypt base.

and lower maltase activities than the control segment.

Animals of 21 days of age normally show transition to postweaned, adult enteric disaccharidase patterns including well-established sucrase activity, decreased lactase and increased maltase activities. Distribution patterns of these enzymes in villus to crypt cell fractions from control jejunal and ileal segments of 21 day old rats are shown in Figure 5. The experimental jejunal segment in continuity, compared with the control segment, shows considerably greater accumulation of sucrase and maltase and generally similar low accumulation patterns of lactase. Exclusion of intraluminal components, from the surgically bypassed ileal segment, had little ultimate suppressive effect on sucrase appearance and accumulation or maltase accumulation in the mature villus cells (both showing apparent greater accumulation than in the control ileal segment). In contrast, however, the bypassed ileal segment continued to retain the preweaned pattern of high lactase accumulation in the villus cells (compare with Figure 4), consistent with the results of the earlier study summarized in Figure 3.

Maturation Delay of Cell Kinetic Changes in Bypassed Ileum

Maturation patterns of rat enteric epithelium include cell kinetic changes, which occur normally coincident with weaning, and involve accelerated proliferative growth, cell migration rate, and turnover time. Ileal bypass, when performed in the preweaned infant rat, results in a maturational delay

in acceleration of proliferative growth, as indicated by a lesser frequency of mitotic cells as shown earlier in Table 1.

That an absence of intraluminal influences may also affect maturational delay in other enteric cell kinetic parameters is illustrated in the experiment summarized in Figure 6. In this study, cell migration and turnover rates were determined on intestinal segments from an experimental animal at 22 days (containing an ileal bypass from 12 days) and control animals of 12 and 22 days of age. The average migration distances of labeled cells along the villus unit are shown (see points of directional arrows) 24 h after labeling accompanying their synthesis in the lower crypt.

In Figure 6A are shown migration distances in 24 h of epithelial cells in comparable jejunal segments from an experimental 22-day-old rat, containing a shortened small intestine in continuity, and from 12- and 22-day-old control rats. Both experimental and control jejunal segments from animals of 22 days of age show similar adult patterns of rapid cell migration, in which new cells formed in the crypt traverse 70% of the length of the crypt to villus column within 24 h. In contrast, cell migration in the jejunal segment of the infant 12-day-old control rat proceeds at seven times lesser rate, indicative of a correspondingly longer cell life-span in the immature intestine.

In Figure 6B are shown average migration distances in 24 h of labeled epithelial cells in com-

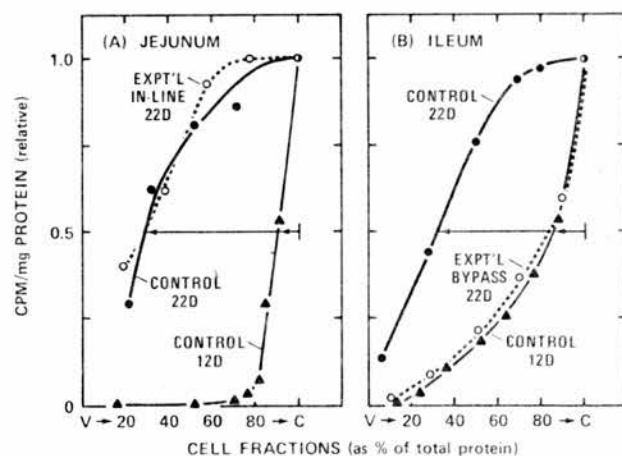


Figure 6. Effect of surgical bypass on migration rate of epithelial cells in A. jejunum of the shortened gut maintained in continuity and B. bypassed ileum. Jejunal and ileal segments were removed from experimental and control rats (see Methods) 24 h after a single injection of [³H]thymidine. A surgical bypass was performed on the experimental animal at 12 days of age. Tissues were removed from the experimental animal at 22 days and from control animals at 12 and 22 days of age for cell migration measurements (see Methods for details).

parable distal segments of bypassed and control ileum from 22-day-old rats and from a 12-day-old control rat. The ileal segment from the control 22-day-old rat, like the jejunal segment, shows an adult pattern of rapid cell migration, in which cells traverse approximately 70% of the crypt to villus column within 24 h. The bypassed ileal segment in the 22-day-old experimental rat, on the other hand, shows cell migration to be maintained at preweaned slower rates, comparable to migration rates found in intestinal segments of the 12-day-old control rat.

Discussion

We have shown that cytokinetic changes, which occur normally in rat intestinal epithelium at the time of weaning, remain suppressed in bypassed ileal segments prepared in the suckling infant. Thus, topical nutrients (mother's milk) and/or alimentary secretions would appear to be involved in their regulation. Interestingly, the expected rapid lactase decline which normally occurs concurrently with cytokinetic changes, fails to develop in the bypassed ileum. The bypassed ileal segment, on the other hand, expresses normal ontogenic developmental changes with respect to sucrase appearance and rapid maltase accumulation.

That fetal intestinal tissue contains an intrinsic, time-related program of sucrase expression, independent of intraluminal influences, has been well documented in studies showing normal developmental sequences of this enzyme in isograft implants of the fetal tissue in kidney capsula of the adult mouse (7) and rat (8). These same studies show, on the other hand, that sucrase expression can be modified by the humoral status of the host with particular reference to cortisone. Whether cytokinetic changes remain suppressed in spite of normal sucrase expression in the isografts was not determined. It is significant, however, that the grafts continued to show high levels of lactase long after the appearance of sucrase (7). That intraluminal influences are involved in enteric lactase decline accompanying weaning is also indicated from recent studies (14) showing that malnourished infant rats continue to retain higher levels of lactase at weaning than well-nourished controls.

In a preliminary report (24), we presented evidence suggesting that abrupt declines in enteric lactase which occur in most mammals at the time of weaning could be the result of coincident reductions in the lifespan of the enterocyte. The hypothesis implies that during postnatal life the rate of lactase synthesis remains generally constant in the differentiated enterocyte, with changes in enzyme concentration at the organ level, reflecting primarily

a change in the average age of the cell population. It is evident from the lactase distribution profiles shown in Figures 4 and 5 that the younger cells emerging from the crypt zone contain less lactase, with accumulation of the enzyme proceeding as the cells age and migrate to the villus tip. Thus the length of time that the cells remain on the villus could provide a primary determinant of enzyme accumulation. We have shown (24) by labeling experiments that lactase synthesis is maintained throughout the life-span of the differentiated enterocyte.

The findings of the present study provide strong additional support for a causal relationship between enteric lactase accumulation and enterocyte life-span. Bypassed ileal segments continue to maintain high levels of lactase at the same time that the remaining intestine maintained in continuity undergoes rapid decline of lactase to low adult levels (see Figure 3). The experimental model system thus allows direct comparison of the relationship between lactase levels and cell life-span in separate intestinal segments of high and low lactase content from the same animal. Analyses from this model demonstrates further the correlative relationship between enteric lactase levels and cell life-span (see Figures 3 and 6), consistent with the hypothesis proposed.

Numerous studies have been reported concerning structural and functional adaptations of enteric epithelium following partial deletion of this organ (by either surgical excision or bypass) in the adult animals (25). The effect of partial deletion on subsequent ontogenic developmental patterns in the small intestine of the infant animal has not been studied previously. Since the surgical bypass employed in the present study results in significant shortening of the intestine in continuity, the adaptive effects on developmental patterns of the disaccharidases were examined.

Precocious appearance of sucrase is evident in the shortened intestine when little enzyme is detectable in either bypassed or control intestinal segments. Early sucrase appearance might result from stimulated cortisol release due to the surgical stress. A similar effect is not observed, however, in the bypassed segment, suggesting specific involvement of an intraluminal factor(s). Both sucrase and maltase accumulate to considerably higher than control levels in the shortened intestine in the 21-day-old animal. The basis for precocious sucrase and maltase response in the shortened intestine is not evident. We could not determine whether differences in nutrient intake (mother's milk) occurred between experimental and control animals. However, since both groups maintained similar body weights, the differences were presumably minimal. Assuming similar nutrient intake, a stimulus would thus be

provided for adaptive change of the shortened intestine in order to process equivalent amounts of food as processed by the control nonshortened intestine. It is significant, however, that lactase undergoes a normal decline to control levels in the shortened intestine, accompanied by accelerated cell migration comparable to the control rate (see Figures 5 and 6).

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